Abstracts accepted for publication only

Pathogenesis

R2084 Frequent detection by real-time PCR of bacteria from the *Helicobacter* and *Campylobacter* genera in stool samples from inflammatory bowel disease patients

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Objectives: Recent studies have suggested that members of the genus *Helicobacter* may play a role in the development of inflammatory bowel disease patients (IBD). The aim of the study was to further investigate this question and to extend it the closely related *Campylobacter* genus.
Methods: Bacteria were detected from stools by real-time PCR based on SYBR-Green fluorescence with an ABI Prism 7000 apparatus (Positivity threshold: Ct < 30). DNA extraction was performed with the QIAamp DNA stool minikit. Four sets of oligonucleotides were used: primers amplifying either a conserved fragment of the 16S rRNA gene from all known species of *Helicobacter* or from all known species of *Campylobacter*; primers specific for a fragment of the vacA gene from *Helicobacter pylori*; and primers specific for a fragment of the 16S rRNA gene from *Campylobacter concisus*. Stools were also cultured for 10 days on Skirrow-supplemented blood agar plates in a microaerophilic atmosphere. 30 patients were included, eight with Crohn's disease (CD), 11 with ulcerative colitis (UC) and 11 symptomatic controls. The association between the presence of *Helicobacter* or *Campylobacter* and each study group was statistically analysed using the Fisher's exact test.
Results: 58% (11/19) of IBD patients [64% of UC patients (7/11) and 50% (4/8) of CD patients] and 27% (3/11) of control patients were *Helicobacter*-positive (ns, p = 0.17). The *H. pylori* PCR was always negative. The *Campylobacter* PCR was positive for 21% (4/19) of IBD patients and 9% (1/11) of control patients (ns, p = 0.46). The *C. concisus* PCR was positive only for the 4 *Campylobacter*-positive patients. 3 of these 4 patients were also *Helicobacter*-positive. Culture yielded only two *C. concisus* strains, from the stools of two of the four patients that were also *C. concisus*-positive by PCR.
Conclusion: Although our results are not statistically significant, they suggest that stools from IBD patients are more frequently *Helicobacter*-positive (but *H. pylori*-negative) than those from control patients. Thus Helicobacteriaceae may have a pathogenic role in the development of IBD. As *C. concisus* was only found in stools from IBD patients, it may also be implicated in IBD.

R2085 Fibronectin binding proteins in *Staphylococcus aureus* strains isolated from 6 to 14-year-old nasal carriers

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Objective: *Staphylococcus aureus* is an important pathogen capable of causing a variety of infections. Nasal carriage rates for *S. aureus* have been reported to be between 18% and 50% in different populations and this carriage represents a risk factor for invasive infections. The aim of the study presented here was to investigate the presence of fibronectin binding proteins (FnBPs) mediating adhesion of *S. aureus* to human epithelial cells.
Methods: Fifty *S. aureus* strains isolated from nasal swab specimens of 6–14 years old healthy children were included in the study. Specimens were inoculated on mannitol salt agar plates and all colonies surrounded by yellow zones on plates after 24–48 hours of incubation at 37ºC were selected. The isolates were identified by biochemical properties and tube coagulase test. Methicillin susceptibility tests of all strains were performed by disk diffusion test using 30 mcg cefoxitin disks and by agar dilution method using oxacilline base according to the recommendations of Clinical and Laboratory Standard Institute (CLSI) guidelines. Presence of FnBPs was investigated by detection of fnbA and fnbB genes via conventional PCR method. *S. aureus* NCTC 8325 was used to characterise the genes coding FnBP A and B as the reference strain.
Results: According to cultural properties and positive tube coagulase test all 50 isolates included in the present study were identified as *S. aureus*. All isolates were found to be susceptible to oxacilline (MIC < 1 mg/L). Of 50 *S. aureus* strains, 14 (28%) were found to be positive for gene fnbA and 5 (10%) for gene fnbB.
Conclusion: Presence of FnBPs in our study population was lower than the other studies performed on nasal carriers published previously. We concluded that the lower age of study population or geographical diversities could be resulted in this lower rate. Fibronectin binding proteins may represent an increased risk factor for subsequent infections but they are not efficient for adhesion alone.

Animal models including experimental treatment

R2086 Effectiveness of albendazol against viability of *Entamoeba histolytica* in experimental animals

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Objective: Intestinal amebiasis is still an important health problem in developing countries of the world. One of the most issues for future biomedical research is the development of antimicrobial resistant, in order to search for alternative new antiamoebic drugs. A study was carried out to evaluate the efficacy of albendazol on the viability of *Entamoeba histolytica* clinical isolate from human which used for experimental animals.
Material and Methods: All experimental animal models (mice and rabbits), divided into 3 groups, each group with either 10 mouse or 10 rabbits, were orally infected with *E. histolytica* (clinical isolate), then after 7 days they were given drugs (Metronidazol or Albendazol) daily according to body weight prepared in advance for 5 days duration and in addition to the controls without drugs. Stool specimens of each group were examined microspically for viable trophozoites, and the number of these trophozoites were counted with haemocytometer chamber, as compared to untreated and treated groups. Statistical methods used was student t-test.
Results: The results showed infection of *E. histolytica* was able to be intiated in rabbits only. Albendazol and metronidazol were highly effective(100%) on treatment of infected groups of rabbits (table I). Trphozoites of *E. histolytica* was highly sensitive to albendazol (25% viability), or to metronidazol (22.7% viability) at a dose of 400 mg/kg/day and 250 mg/kg/day respectively, which was significant in relation to the control 500% viability (table II). However, the differences were significant at the level ($p < 0.01$).
Conclusions: The presenstudy showed that the newly used albendazol is very effective anti-amebic drug as metronidazol in rabbits.

R2087 The effect of artesunate on *Toxoplasma gondii*: in vitro and in vivo studies

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Objectives: In the search for new effective compound with less toxic effects for treating *Toxoplasma* infection, this study was conducted to investigate the effect of AS on *Toxoplasma gondii* (*T. gondii*) both in vitro and in vivo.

Methods: In the in vitro study, tachyzoites of RH strain of *T. gondii* were exposed to AS in a concentration of 2 μg/ml for 72 hours. The assessment of the effect of AS was carried out by studying; the viability, infectivity and ultrastructure changes of the treated tachyzoites by scanning electron microscope (SEM). In the in vivo study, Swiss albino mice were infected intraperitoneally with tachyzoites of RH strain of *T. gondii* then orally treated with AS in a dose of 200 mg/kg for five successive days. The effect of AS was evaluated by detecting the mortality rate and the survival time of the infected treated mice. Parasite burden, viability, infectivity and ultrastructure changes of tachyzoites harvested from the peritoneal cavities of infected treated mice, in comparison with that of infected non-treated control animals, were also studied.
Results: Results of the in vitro study demonstrated a significant reduction in the viability and infectivity of tachyzoites exposed to AS as compared with the untreated controls. Regarding the in vivo study, treatment of mice with AS induced a significant decrease in their mortality rate and increase in their survival time. There was also a significant reduction in the parasite burden in the infected treated mice associated with significant reduction in viability and infectivity of tachyzoites harvested from their peritoneal cavities as compared with the infected non-treated control. The SEM study demonstrated distortion in the tachyzoites' shape, peeling, erosions and discontinuity in areas of the surface membrane of the treated tachyzoites of both in vitro and in vivo studies.
Conclusion: The results of the present study suggested that, AS could provide an effective and promising drug in treating acute toxoplasmosis. This will open the way to study its effect on cyst forming strains of *T. gondii* and to determine its efficacy and safety in treating toxoplasmosis in humans.

Biofilm

R2088 Effects of extremely low-frequency electromagnetic fields on *Helicobacter pylori* biofilm

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Objective: *Helicobacter pylori* is characterised by a dynamic behaviour in response to environmental stress entering the viable but not culturable (VBNC) state in which the microorganism modifies its morphology from spiral to coccoid form with a loss of culturability. This cellular response is emphasized when bacterial cells organise themselves into microbial communities forming biofilm.
The aim of this work was to investigate the effect of exposure to an extremely-low frequency electromagnetic field (ELF-EMF), able to interfere on phenotypic and transcriptional prokaryotic pattern, both in the formation and in the detachment of the *H. pylori* biofilm.
Methods: The reference strain *H. pylori* ATCC43629 was used for this study. Bacterial cultures grown on polystyrene surfaces were exposed at 50Hz of frequency and 1 mT of magnetic field within a central area of a cylindrical solenoid and checked over time. The exposed cultures and the respective sham-exposed controls were studied for: the cell viability status; the cell morphological aspects; the biomass measurement; the analysis of DNA fingerprintings and the expression of genes coding for hsp60 and amiA involved in the bacillary/coccoid form conversion.
Results: The ELF-EMF effect was studied on 2 day sessile *H. pylori* cells and on cultures first grown as biofilm and then exposed for 2 days for a total time of 4 days.
Cultures exposed for 2 days displayed significant differences on cell viability as well as on bacterial morphology with the prevalence of bacillary forms (58.41%) in respect to the unexposed ones (33.14%). When comparing the 4 days cultures, significant differences were not found. The measurement of biofilm cell mass displayed, in both examined experimental condition, a significant reduction of adhesion in exposed cultures at 2 (from 0.0183±0.060 to 0.0067±0.0064 OD492) and 4 (from 0.2759±0.0678 to 0.1472±0.0394 OD492) days. No changes on DNA fingerprintings were recorded whereas a modulation in hsp60 and amiA expression among exposed and unexposed cultures were expressed.
Conclusion: Our results indicate that the exposure to 50Hz EMF of *H. pylori* biofilm induces phenotypical changes on adherent bacteria and produces a reduction on cell adhesion also suggesting a possible role in the variability into *H. pylori* population.

R2089 In vitro comparison of anti-biofilm effects against carbapenem-resistant *A. baumannii*: imipenem, colistin, tigecycline, rifampicin and combined regimens

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Background: Multi-drug resistant (MDR) *Acinetobacter baumannii* has emerged as one of the most important nosocomial pathogens. In addition to the diverse resistance mechanisms, some *A. baumannii* strains are known to have biofilm-producing capacity, thereby decreasing antibiotic effectiveness.
Methods: This study was designed to assess biofilm-producing capacity of three different MDR *A. baumannii* strains with diverse resistance mechanisms (OXA-51, IMP-1 and VIM-2 type β-lactamases), and intended to compare the effect of each antibiotic regimen (rifampicin, colistin, imipenem, tigecycline, rifampicin-imipenem and rifampicin-colistin) on mature *A. baumannii* biofilms using in vitro polystyrene plate biofilm assay.
Results: Among three MDR *A. baumannii* strains, only VIM-2 strain produced strong biofilm compared to the controls (optical density, 8.04±2.16 vs. 0.49±0.26). Regarding VIM-2 strains, neither imipenem, colistin, nor rifampicin reduced biofilm formation alone at MIC of each antibiotic agent (inhibition of biofilm synthesis less than 30%). In comparison, tigecyclin (0.76±0.23), imipenem-rifampicin (1.07±0.31) and colistin-rifampicin (1.47±0.54) showed a significant inhibition of biofilm synthesis compared to the positive controls at 48 hours after incubation ($p < 0.01$). Tigecycline inhibited biofilm formation even at the one fourth level of MIC (1.17±0.21). Likewise, both imipenem and colistin were also effective at the reduced concentrations when those were combined with rifampicin. Such biofilm-inhibiting effects with those antibiotic regimens sustained up to 96 hours after incubation.
Conclusions: VIM-2 *A. baumannii* strain was a strong biofilm producer. Tigecycline, imipenem-rifampicin and colistin-rifampicin would be effective against diverse infections by biofilm-producing *A. baumannii* strains.

R2090 Comparison of different methods for quantification of *Candida* spp. biofilm grown in different glucose concentration

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Objectives: *Candida* species are emerging as important pathogens. In this study biofilm formation among invasive and non-invasive *Candida* isolates using different quantification methods as well as different glucose concentrations was evaluated.
Material and Methods: Two hundred-and-thirty-four *Candida* isolates including 160 *C. albicans*, and 74 non-*albicans* isolates were recovered from bloodstream (106), skin swab (40), pharyngeal swab (30), urine (35), bronchial fluid (11), and medical devices (12). In 96-well microtiter plates biofilm production was studied using as growth medium (GM) RPMI 1640 medium containing three different glucose concentrations: 0.2%, 2%, and 8%. *Candida* biofilms were quantified using three different methods: biofilm thickness by the percent transmittance (% T) method expressed as %Tbloc, metabolic activity by XTT assay expressed as optical density (OD), and biofilm mass by crystal violet staining expressed as OD. Using values assessed by % T method biofilm production was scored as negative, low or high, and compared with metabolic activity and biofilm mass.
Results: Biofilm production was more frequently observed in GM containing 2% and 8% glucose (230 of 234 isolates, 98.3%) than in GM containing 0.2% glucose (212 of 234 isolates, 90.6%). In non invasive isolates biofilm production was more commonly detected than in invasive

isolates: 96.9 vs. 82%, $p < 0.001$ (in GM containing 0.2% glucose), and 99.2% vs. 96.2%, $p > 0.05$ (in GM containing 2% and 8% glucose). Correlation between biofilm production assessed by % T method and biofilm mass assessed by crystal violet staining was noticed: non biofilm producers (OD $\geqslant$ 0.1), low biofilm producers (OD 0.1–0.25) and high biofilm producers (OD $\geqslant$ 0.25). The OD of biofilm formation assessed by XTT assay correlated with values assessed by other two assays in all *Candida* spp., except for *C. tropicalis* and *C. glabrata*, by which OD decreased in GM containing 2% and 8% glucose.

Conclusion: Our data suggest that RPMI 1640 medium containing 2% and 8% glucose support the highest biofilm production. For the quantification of biofilm formation XTT assay showed some limitation, particularly in *C. glabrata* and *C. tropicalis*.

R2091 Differences between *Pseudomonas aeruginosa* non-adapted and adapted to benzalkonium chloride: comparison of adhesion capacity, and susceptibility of biofilms to removal and surfactant attack

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Bacteria adhesion, and consequent biofilm formation, in vitro and in vivo, are phenomena that often occur naturally but are also bacteria's strategies to protect themselves from stress factors, playing probably an important role in virulence. Furthermore, bacteria growing in biofilms are less susceptible to many antibacterial agents than their suspended counterparts. These factors emphasize the need of suitable and efficient surface disinfection procedures in order to reduce the overgrowth of resistant microorganisms in response to an ineffective course of antimicrobials. In this study, we examined the effect of adaptation of *P. aeruginosa* to a surfactant, benzalkonium chloride (BZK), on the adherence of bacterial cells to PET, conditioned and non-conditioned with BZK, and on their ability to resist to removal and BZK aggression. The assays were carried out in a PPFC. Bacterial adaptation was attained by exposing *P. aeruginosa* to gradual increasing concentrations of BZK, and selected in TSA supplemented with 4.0 mM BZK.

The results show that adapted *P. aeruginosa* adhered in a more extent than the non-adapted counterpart. For both strains, the pre-conditioning of the PET surfaces significantly favoured bacterial adhesion. The higher adhesion was observed with the adapted bacteria onto the conditioned PET coupons. These results highlight that the extent of adhesion is greater the higher are the stress factors. The strength of adhesion is also higher in the case of adapted bacteria since detachment only occurs with *P. aeruginosa* non-adapted. BZK application did not cause significant removal except for *P. aeruginosa* non-adapted adhered to non-conditioned PET. Nevertheless, BZK attack causes loss of viability of the cells that remained adhered to the surfaces, this loss being more notorious in the case of non adapted cells adhered in the conditioned surfaces.

Based on the results it can be said that the presence of BZK residues on the adhesion surfaces did not impair the bacterial adhesion capacity though affects the viability of the adhered cells. It can also be concluded that resistant bacteria that survived to a simple adaptation step to a common antimicrobial agent increased its adherence ability and insusceptibility to removal and antimicrobial treatment. In a disinfection point of view, these results can represent additional problems for the eradication of pathogenic bacteria with increased virulence from equipment and surfaces in the medical arenas.

Antimicrobial pharmacokinetics, pharmacodynamics & general pharmacology

R2092 Killing kinetics of cefditoren pivoxil against *Escherichia coli* clinical isolates

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Objectives: Cefditoren pivoxil (CFD), an advanced oral cephalosporin is licensed for urinary tract infections (UTI) only in Japan. In view of the increasing resistance in community infections, this study aims to evaluate the in vitro efficacy and killing kinetic profile of CFD against recently isolated uropathogens from Greece.

Methods: The susceptibility of *Escherichia coli* isolates recovered during 2005–2007 from outpatients with UTIs, was tested against 17 antimicrobials with disk diffusion method and for CFD with agar dilution method (CLSI 2007). In lack of official breakpoints, isolates with MIC $\geqslant$ 2 mg/L were considered as non-susceptible (NS) according to recent literature. The killing kinetic profile of CFD was also explored with killing curves for 18 isolates of *E. coli* (16 susceptible, 2 non-susceptible), using concentrations of 1×, 2× and 4×MIC and within 0 h, 2 h, 4 h, 6 h and 24 h of incubation.

Results: The CFD MIC50/90 values of 332 *E. coli* isolates were 0.25/0.5 mg/L (range 0.06–>32 mg/L). Resistance rates among commonly used antimicrobials and CFD were: ampicillin 31.9%, amoxicillin/clavulanate 6.6%, ciprofloxacin 4.2%, co-trimoxazole 23.8%, cefuroxime axetil 3.9%, nitrofurantoin 6.3% and CFD 3%. Killing curves showed that the bactericidal end point ($\geqslant$3 log10 reduction) was reached for 1×MIC concentration within 4 h for 1 isolate and within 6 h for 2 isolates. For 2×MIC, killing was observed within 4 h for 2 isolates, within 6 h for 6 isolates and within 24 h for 5 isolates(1 of them NS) and for 4×MIC within 4 h for 1 isolate, within 6 h for 12 isolates and within 24 h for 4 isolates (1 of them NS). For 1 NS isolate there was no killing effect.

Conclusion: This study demonstrated the in vitro efficacy and bactericidal activity of CFD in recently isolated uropathogens from outpatients, thus indicating a possible future role as an alternative in UTIs treated in outpatient settings, or for patients receiving sequential iv/oral treatment.

R2093 Steady-state plasma and tissue concentrations of tigecycline after intravenous infusion administration to rabbits

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Background: Tigecyclin (T) is the first available glycycycline derived from minocycline. It possesses broad-spectrum activity against aerobic and anaerobic bacteria including multidrug resistant Gram positive and Gram negative pathogens.

Trigecyclin was found to exhibit large volume of distribution in healthy humans, indicating extensive tissue distribution and half-life elimination of 37 to 67 hours. With a single intravenous (IV) dose of 7 or 14 mg/kg in rabbits the drug concentration in serum can remain above the MIC for more than 12 hours.

Objectives: To measure T accumulation to body tissues at steady state in order to construct a full body physiological based PK model and to describe drug disposition kinetics in rabbits.

Methods: We used New Zealand white rabbits (3.0±0.4 kg). After a loading IV dose of 75 mg T in 15 min, animals received 27.5 mg of T during a 2.5 h continuous IV infusion to achieve a 2 μg/mL plasma steady state concentration (SSC). Blood sample measurement at 60, 45, 30 and 15 min prior and immediately after the end of the 2.5 h IV infusion were used to confirm SSC. At the end of the 2.5 h IV infusion all rabbits were anaesthetized and sacrificed. Tissue samples were taken to determine drug accumulation. Blood samples were centrifuged at 4000 rpm for 15 min and plasma samples were frozen at −70ºC until analyzed. Tigecycline assay was performed by a HPLC-UV method recently developed in our laboratory and properly modified for the needs of this study.

Organ	Concentration (μg/g)	Organ	Concentration (μg/g)
Heart	7.83 (±3.4)	Peritoneal fat	1.6 (±0.5)
Spleen	11.3 (±2.2)	Orbital fat	4.8 (±2.2)
Liver	38.5 (±16.4)	Bone marrow	0.3 (±0.1)
Kidneys	20.9 (±9.0)	Brain	0.3 (±0.03)
Gallbladder	70.1 (±21.5)	Lung	7.8 (±2.0)
Bile	200.0 (±80.5)	Testicular tissue	6.8 (±1.4)
Thigh muscle	10.4 (±4.4)	Vitreous fluid	0.4 (±0.1)

Results: The result are presented in the table.

Conclusion: Results show that in rabbits T accumulates to well perfused tissues such as kidneys, liver, spleen, muscle and testicular tissue. Due to its hydrophilic nature tigecycline concentration in fat tissues is low and is not distributed in the brain.

R2094 Extended studies of piperacillin/tazobactam generic formulations: variations of branded product lots and assessment of 38 generic lots

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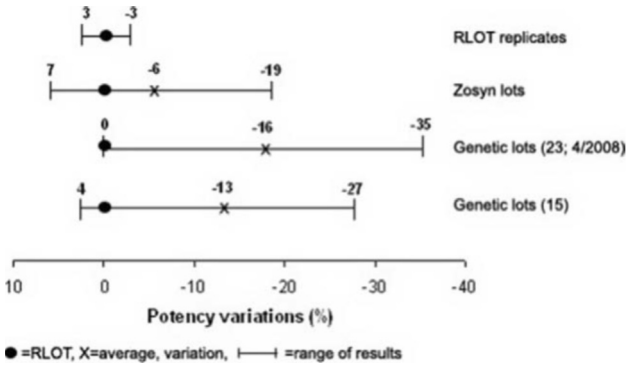
Objectives: To further assess the potency of piperacillin/tazobactam (P/T; Zosyn®, Wyeth) branded and generic lots produced for global sale. P/T is a widely used broad-spectrum β-lactamase inhibitor combination, and a large number of generic products are available needing careful evaluation/comparison to the reformulated branded product.
Methods: Updated analysis was performed in three parts; 1.) MIC assay variations using reference (RLOT) branded lot (B75011; 13 replicates over 2 years); 2.) other branded lots compared to the RLOT (8 total); and 3.) expanded tests of 38 generic lots (23 reported earlier). Disk diffusion and MIC assays used 4 organisms (P/T MIC, ranges of 1 to 4 mg/L), with triplicate processing compared to the RLOT potency control. A total of 24 manufacturers of generic P/T were evaluated from 13 nations. Assay susceptibility tests employed the CLSI M7-A7 method with 20 incremental dilutions steps between 0.5 and 8 mg/L, increasing precision. Only MIC assays are presented.
Results: Replicate tests (4 organisms × triplicate tests × 13 testing events) of RLOT showed consistent results only varying ±3% from averaged MIC results with each assay strain (see figure). When other P/T branded lots were tested, the potencies varied from +7 to −19% (average at −6%). This compares to generic lots (Figure) exhibiting potency variations of +4 to −27% (average, −13%) in recent testing and equal to −35% in year 2007 assays. Only 6 of 38 (16%) generic lots had a potency at least equal to the average of all Zosyn® branded lots. All values were based on demonstrated antimicrobial activity of vial contents, where only one generic lot (3%) had activity >RLOT.
Conclusions: The activity of P/T generic products can vary and significant decreases (−15% overall) have been noted by this precise incremental MIC assay system, RLOT test variations were minor and the branded lots had greater activity correlations (−6%) when compared to results from 38 generic lots (Figure). Selection of generic products should consider the quality of the formulation as measured by in vitro potency as well as chemical-based analyses.



R2095 Antimicrobial effect of polysaccharide extract of *Ganoderma* on some pathogen microorganisms

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Objective: Antimicrobial activity is the ability of a substance to inhibit preferably; or to kill microorganisms. Due to emergence of resistance to antibiotics amongst microorganisms, investigations for novel antimicrobial agents have always been one of the major pre-occupations of the medical society. Mushroom is a common name for the fruiting body of macro fungi particularly to the members of Agaricales, edible as well as highly poisonous species. Medicinal mushrooms have been used in folk medicine throughout the world since the ancient times. The present investigation aims to study the antimicrobial activity of medicinal mushroom namely *Ganoderma* Reishi.
Materials and Methods: In this present work, *Candida albicans* MTCC 1637, *Klebsiella* pneumonia MTCC 432, and *Staphylococcus aureus* HAL 2079, were used as test microorganisms to test polysaccharide extraction of *Ganoderma* for the antimicrobial activity with a well assay method and determination minimum inhibitory concentration by standard tube agglutination.
Results: The crude polysaccharide extract of *Ganoderma* Reishi (3a & 3b) was active against *Candida albicans* (MTCC 1637), *Klebsiella* pneumonia (MTCC 432), and *Staphylococcus aureus* (HAL 2079) while, Polysaccharide fraction (3a) showed the zone of inhibition 28, 22 & 25 mm and MIC 32, 32 and 64 and polysaccharide fraction (3b) showed zone of inhibition 35, 20, & 27 mm and MIC 32, 64 and 64 in cases of *Candida albicans*, *Klebsiella* pneumonia & *Staphylococcus aureus* respectively.
The maximum zone of inhibition was found to be 35 mm in standard antibiotics while 34 mm was observed in the present study.
Conclusion: This data clearly indicates that the mushroom compounds show equivalent compatibility with the standard antibiotics. However, further separation and fraction of polysaccharide fractions need to be carried out to detective the bioactivity of specific compounds.

Mechanisms of action and resistance

R2096 Molecular analysis of isoniazid resistance in different genotypes of *Mycobacterium tuberculosis* isolates from Iran

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Objectives: Significant increase of isoniazid-resistance in Iranian *Mycobacterium tuberculosis* isolates in the last few years would present a serious need for rapid detection and effective management of INH-resistance tuberculosis in Iran. Therefore the aim of present study was to investigate the prevalence of mutations in the three most commonly reported loci associated with INH-resistance, katG codon 315, the fabG1-inhA regulatory region and oxyR-ahpC intergenic region.
Methods: To investigate the mutations associated with isoniazid resistance, Drug susceptibility test was performed initially and then, parts of the coding sequence of katG gene and fabG1-inhA and oxyR-ahpC regulatory regions, were analyzed in a sample of 48 isoniazid-resistant and 25 isoniazid-sensitive isolates using nucleotide sequencing.
Results: The R463L polymorphism in katG gene was detected with high frequency in both Isoniazid resistant and sensitive isolates. The ahpC 46A was the most common mutation in the oxyR-ahpC intergenic region which was present in 31.2% of resistant and 16.0% of susceptible isolates. Mutations at katG codon 315 or the fabG1-inhA regulatory region were identified in 77.0% of the isoniazid-resistant isolates. Spoligotyping and IS6110 RFLP patterns revealed that most of the isolates contained ahpC 46A and katG 463Leu polymorphism belonged to CAS super family.
Conclusion: mutations at katG codon 315 or the fabG1-inhA regulatory region were identified in 77.0% of the isoniazid-resistant isolates and in none of the isoniazid-sensitive strains and are highly predictive of isonizid resistance in Iranian isolates.

R2097 The accumulation of resistance mutations in *Mycobacterium tuberculosis* in vitro

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Objectives: In *Mycobacterium tuberculosis* (MTB), antibiotic resistance is almost exclusively established by point mutations. Multidrug-resistance develops by sequential acquisition of mutations at different

loci. Although the acquisition of resistance mutations is random, the genetic routes leading to the development of successful and virulent (multi)drug-resistant bacteria may be quite constrained (Weinreich DM et al. Science 2006) and probably influenced by the genetic background of the strain in question.
We have studied the acquisition of drug resistance mutations in different strains and isogenic mutants to better understand the development of MDR strains.
Methods: Cultures of well-characterised laboratory derived isogenic mutants and clinical isolates were used. Drug-resistant mutants were selected in vitro from either a single large liquid culture or a series of smaller liquid cultures by plating on solid antibiotic-containing medium and mutant colonies characterised using a multiplex ligation probe assay (MLPA) and DNA sequencing.
Results: Different clades have a preference for different distributions of specific rpoB mutations, this effect was most strongly evident when individual colonies were selected from a single large broth (frequency). Less clade specific variation was seen with multiple cultures(rate). Most notably we detected a marked increased in the proportion of rpoB C526T mutations (typically greater than 50%) from strain Mtb72(Group 3 genotype) when selected from a single large broth.
Initial results indicate a possible reduction in the diversity of rpoB resistance genotypes after selection of strains with pre-existing INH resistance.
Conclusion: Strain specific variation in favoured mutations was more evident when multiple colonies are characterised from a single large culture. We believe this indicates that the relative fitness of the different mutations varies based on the strain's genotype. Our results for the variation in distribution of spontaneous rpoB mutations between different clades shows similarity to variations in the distribution of mutations previously reported from clinical isolates within genotypic groups (Lipin MY et al. Clin Microbiol 2007). These results suggest the sequence of mutations leading to a successful MDR strain may indeed be quite constrained with certain mutations in certain genotypes probably being significantly more likely to become multiply resistant.

R2098 Adhesion and adhesion inhibition properties of *Bifidobacterium* strains *B. longum* BB536 and *B. pseudocatenulatum* G4 on HT-29 epithelium cell line

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Objective: To investigate the adhesion and adhesion inhibition properties of *Bifidobacterium* strains *B. pseudocatenulatum* G4 and *B. longum* BB536 to the model intestinal epithelium consisting of HT-29 cell-line under different pH levels at different exposure times.
Methods: Adhesion ability of two *Bifidobacterium* strains *B. longum* BB536 and *B. pseudocatenulatum* G4 was evaluated using HT-29 human epithelium cell line in vitro. Four different levels of pH were used 5.6, 5.7, 6.6, and 6.8 with two different times 60 and 120 min. Adhesion was quantified by counting the adhering bacteria after Gram staining. The evaluation ability of *B. longum* BB536 and *B. pseudocatenulatum* G4 to inhibit adhesion of selective pathogens was done.
Results: The highest adhesion capacity for both *Bifidobacterium* strains was observed at 120 min, the maximum adhesion capacities for both strains were at pH level 5.7. *B. longum* BB536 and *B. pseudocatenulatum* G4 showed the ability to compete and inhibit adhesion of pathogens. The ability to inhibit the adhesion or competitive to adhere pathogens was variable depending on both probiotic and pathogen tested.
Conclusion: Our results indicate that the ability to adhere and inhibit adhesion of pathogens can be used for preliminary screening in order to identify potentially probiotic bacteria suitable for human or animal consumption.

R2099 High prevalence of mefE gene among macrolide-resistant *Streptococcus pneumoniae* isolates in Istanbul

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Objective: Macrolide resistance due to antibiotic efflux in *Streptococcus pneumoniae* is getting increased in many parts of the world. Macrolide efflux pump encoded by either mefA or mefE genes confers low-level resistance to 14- and 15-membered macrolides but not to 16-membered macrolides, lincosamids, or streptogramin B.
We aimed to determine the prevalence of erythromycin resistance and the role of mef directed efflux pump in *S. pneumoniae* strains isolated in our university hospital during 2008.
Methods: The prevalence of erythromycine resistance have been determined by disk diffusion method in 91 *S. pneumoniae* isolates. Broth microdilution method was applied in detection of erythromycin and clindamycin MICs of the pneumococcal strains which were found to be resistant to erythromycin in disk diffusion test. Two major macrolide resistance mechanisms regarding efflux and target modification were investigated in erythromycin resistant *S. pneumoniae* isolates, phenotypically and genotypically. Double disc method was used to determine the macrolide resistance phenotypes (cMLSB, iMLSB and M phenotype). The presence of mef genes and ermB genes were analysed by PCR using primers that distinguish mefA and mefE genes.
Results: Resistance to erythromycin was detected in 28.6% (n:26) of the isolates. Erythromycin MICs ranged from 2 μg/ml to ⩾512 μg/mL and MIC50 of the resistant strains was ⩾512 μg/mL. Twenty-four of erythromycin resistant isolates were in cMLSB phenotype and 2 were in M phenotype (Table 1).

Table 1. Phenotypic and genotypic test results in erythromycin resistant *S. pneumoniae* isolates

Resistance phenotype	No. of isolates (%)	MIC range (μg/mL)*		Resistance genotype no. of isolates (%)		
		Erythromycin	Clindamycin	*erm*(B)	*mef*(E)	*erm*(B)+*mef*(E)
$cMLS_B$	24 (92.3)	128–⩾512	⩾512	10 (38)	0	14 (53.8)
M	2 (7.7)	2–4	0.125–0.25	0	2 (8)	0

*Determined by broth microdilution recommended by the CLSI.

In the isolates representing M phenotype, erytromycin MICs remained in low level (2–4 μg/mL) and their clindamycin MICs were in susceptibile range. Of the 26 erytromycin resistant strains, 14 (53.8%) were found to harbour mefE and ermB genes together; whereas 2 (7.7%) of them harboured mefE alone.
Conclusion: This is the first report indicating the high prevalence of mefE gene together with ermB gene in macrolide resistant *S. pneumoniae* isolates in Turkey. Although target modification (ErmB) was determined as the mechanism responsible of high level erythromycin resistance in our strains; the presence of mefE gene either alone or together with ermB in these isolates signifies a new threat in macrolide resistance of *S. pneumoniae* in our region.

R2100 Molecular mechanisms of macrolide resistance in invasive *Streptococcus pneumoniae* isolated from Thai patients

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Objectives: Macrolide resistance in *Streptococcus pneumoniae* is an increasing problem worldwide. Two main mechanisms of macrolide resistance are active efflux, encoded by the mef(A) gene and methylation of antibiotic target site, encoded by the erm(B) gene. Other mechanisms of resistance include mutations in 23S rRNA and ribosomal proteins L4 and L22. The aim of this study was to investigate the prevalence and molecular mechanisms of macrolide resistance in invasive *S. pneumoniae* isolated from Thai patients.
Methods: A total of 100 invasive *S. pneumoniae* isolates were obtained from patients at King Chulalongkorn Memorial Hospital, Bangkok, Thailand between February 2001 and March 2006. The minimal inhibitory concentrations (MICs) of erythromycin, clarithromycin and

penicillin were examined by agar dilution and Etest. All macrolide-resistant isolates were investigated for the presence of mef(A) and erm(B) by PCR. Mutations in the genes encoding domain V of the four copies of 23S rRNA and ribosomal proteins L4 and L22 were determined by PCR and DNA sequencing.
Results: Of the 100 invasive *S. pneumoniae* isolates, 36% were resistant to erythromycin, 34% to clarithromycin and 16% to penicillin. Erythromycin resistance was found in 5.7% (3/53) of penicillin-susceptible isolates, 64.5% (20/31) of penicillin-intermediate isolates and 81.2% (13/16) of penicillin-resistant isolates. Of the 36 erythromycin-resistant isolates, 12 (33.3%) carried mef(A) and 24 (66.7%) carried erm(B). Sequencing analysis revealed alteration in ribosomal protein L4 at Ser20 to Asn in 36.1% (13/36). No mutations were detected in the four copies of the 23S rRNA genes and ribosomal protein L22.
Conclusions: This study demonstrates high prevalence of macrolide resistance in invasive *S. pneumoniae* isolated from Thai patients and the principal resistance mechanism is mediated by a 23S rRNA methylase, encoded by erm(B).

R2101 Mechanisms of macrolide resistance in *Streptococcus agalactiae* isolated at a Tunis hospital (2005–2007)

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603 non duplicate clinical *Streptococcus agalactiae* were collected. 155 (25.7%) were resistant to erythromycin. The isolates were identified by conventional methods and specific agglutination grouping. Antibiotics susceptibility testing was done using disk diffusion method. A double disk test using erythromycin and clindamycin was used to identify the phenotypic macrolide resistance mechanisms. MICs were determined by E-test for erythromycin, clarithromycin, clindamycin, azithromycin, quinupristin-dalfopristin and linezolid. ermB, ermTR and mefA genes conferring resistance to macrolides were identified by multiplex PCR. Most erythromycin resistant isolates were recovered from urines (38.7%) and vaginal specimens (22.5%). 83.8% showed constitutive MLSB phenotype (MIC50/MIC90: >256/>256 μg/mL for erythromycin and clindamycin), 8.45% inducible MLSB phenotype (MIC50/MIC90: 192/>256 μg/mL for erythromycin and 2/8 μg/mL for clindamycin) and 7.75% M phenotype (MIC50/MIC90: 16/32 μg/mL for erythromycin and 0.5/0.75 μg/mL for clindamycin). Linezolid and quinupristin-dalfopristin showed excellent activity (MIC90: 0.38 and 0.5 μg/mL respectively). Neither resistance to β-lactam nor high level resistance to aminoglycosides was found. Strains with MLSB phenotype harboured ermB gene in 82%, ermTR gene in 8.38% and ermB+mefA genes in 1.87%. All strains categorised as M phenotype carried the mefA gene in 7.75%.
Erythromycin resistance in *S. agalactiae* has reached an important rate in our hospital. MLSB constitutive phenotype conferring cross-resistance to macrolides, lincosamides and streptogramin B with high level of resistance was the most prevalent phenotype. Thus, linezolid and quinupristin-dalfopristin proved to be highly active.

R2102 Susceptibility profiles and detection of resistance genes of carbapenems and 5-nitroimidazole among *Bacteroides* spp. in Turkey

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Background: *Bacteroides fragilis* group (BFG) is the most commonly encountered bacteria from anaerobic infections and more resistant to antmicrobial agents than the other anaerobes. However carbapenems and nitroimidazoles are the most useful antibiotics against *Bacteroides*, resistant isolates have been reported. The genes, which may be responsible for the resistance against carbapenems and metronidazole, are carbapenems (cfiA) and 5-nitroimidazole (nim), respectively. Resistance genes should be activated by upstream insertion sequence (IS) elements such as IS1169, IS1170, IS1186, and IS1187.
Objective: This study investigated the cfiA, nim genes and related IS elements, and determined susceptibility profiles of BFG against carbapenems and metronidazole.
Methods: Different clinical specimens were obtained at Marmara University Hospital. The specimens were collected, transported, and processed as outlined in the Wadsworth-KTL Anaerobic Bacteriology Manual. The strains were identified by using a combination of conventional tests and the commercially available biochemical kits. Antimicrobial susceptibility tests against imipenem, meropenem and 5-nitroimidazole were performed according to recommendations of CLSI (M 11-A7) agar dilution methods. The resistance genes (cfiA, nim) and IS elements were determined by PCR.
Results: Total of 66 BFG strains were isolated and identified as 48 *B. fragilis*, 10 *B. thetaiotaomicron*, 6 *B. uniformis/ovatus*, 1 *B. distasonis* and 1 *B. vulgatus*. There were no resistant strains against metronidazole. However non-*B. fragilis* strains were susceptible to carbapenems, *B. fragilis* strains have 8% resistance rates for imipenem and 10% for meropenem. Metronidazole resistance genes were not detected among all strains. Only *B. fragilis* were positive for cfiA (n:18) gene or IS1187 (n:23) elements. Four strains of *B. fragilis* which have cfiA and IS1187 elements together (n:5) were resistant to carbapenems.
Conclusions: Our findings form a database about resistance genes of pathogenic BFG in Turkey, where molecular investigation of antimicrobial resistance of anaerobes has not been performed so far. For the present, it looks like there was no risk for metronidazole resistance. Although non-*B. fragilis* strains are susceptible to carbapenems, nearly 10% of *B. fragilis* strains are resistant to them. Because of possessing cfiA gene and IS elements is more common among *B. fragilis*, it seems to be important to monitor *B. fragilis* for emergence of resistant strains.

Resistance surveillance

R2103 MRSA in Venezuela, 20 years of history

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Background: MRSA is a worldwide risk for patients in hospitals and, more recently in community acquired infections.
Venezuela has a national surveillance program for bacterial resistance since 1988. We present here the historic evolution of *S. aureus* resistance in our country.
Methods: All *Staphylococcus aureus* isolated in 49 laboratories around the country were tested by disk diffusion using CLSI criteria or Vitek system. The Whonet software was used for prospective data entry and reports. For data management the Epi Info 3.4.3 (CDC) and Language for Statistic Programs R version 2.5.1 (The R Foundation for Statistical computing) were used.
Results: A total of 262635 *S. aureus* were identified during last 20 years, 60% from inpatients and 40% from outpatients.
The MRSA rate was 14% in 1988 in inpatients. This rate remained stable for years up to 2002 (15%). Since 2003, an important increase in prevalence of MRSA was observed, climbing up to 45% in year 2006. The proportion of MRSA from the community, also increased up to 26% in year 2006.
Conclusion: From 1988 to 2002 Venezuela had a low and stable rate of MRSA, since then a high increased have seen. The reasons for these increase are no clear. The presence of Chilean clone has been observed.

R2104 Vascular catheter infection pattern. A temporal surveillance study of 581 consecutive episodes in a tertiary-care hospital

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Introduction: A prospective microbiological surveillance program is ongoing at our tertiary-care Hospital located in Northern Italy.
Patients-Methods: The trend of microbial isolations from patients admitted during the last calendar year (January to December 2007), with

a clinically- and microbiologically-confirmed central venous catheter (CVC) infection, is regularly reported on quarterly basis.
Results: The trend of CVC infections monitored among our inpatients moderately varied during the observation period (149 cases in January-March, 169 episodes in April-June, 129 cases in July-September, and 134 episodes in October-December). Among the most frequent organisms, *Staphylococcus epidermidis* accounted for the majority of isolates (183 cases: 31.5%), followed by *Escherichia coli* (49: 8.4%), *Staphylococcus aureus* (45: 7.7%), *Pseudomonas aeruginosa* (36: 6.2%), *Enterococcus faecalis* (30: 5.2%), *Enterococcus faecium* (25: 4.3%), *Klebsiella pneumoniae* (21: 3.6%), and *Enterobacter cloacae* (15: 2.6%), while the yeast *Candida albicans* accounted for a minority of episodes (17 only: 2.9%). When analysing the available figures according to calendar months, only some Gram-negative pathogens showed an increasing incidence over time: *Pseudomonas aeruginosa* from 5.4% in the first three months of 2007 up to 7.5% in the last three months of 2007, and *Enterobacter cloacae* (from 2.0% in January-March 2007, up to 2.68% in October-December 2007), as well as other environmental Gram-negative organisms.
Conclusions: A prospective microbiological monitoring may notably add to the knowledge of local epidemiological figures and antimicrobial sensitivity trends of CVC infection (which represent relevant causes of hospital-related morbidity), and plays a highly significant role in the selection and planning of chemoprophylactic and therapeutic choices, on both local and regional settings. Although the major causative agents of CVC-related infection among hospitalised patients remain staphylococci as a group, however the progressive emerging of Gram-negative pathogens is appreciable also over a proportionally short (12-month) observation period, and deserves major attention by Microbiologists and Clinicians.

R2105 Surveillance of antimicrobial resistance and serotype epidemiology of *Streptococcus pneumoniae* in Crete, Greece

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Objective: To determine the antimicrobial resistance and seroprevalence in clinical isolates of *Streptococcus pneumoniae* collected from patients at the University Hospital of Heraklion, Crete, Greece, during the years 2000–2007.
Methods: A total of 417 clinical isolates of *Streptococcus pneumoniae* collected over a 7-year period, were studied. Antimicrobial susceptibility testing was performed by the E-test method and the results were interpreted following CLSI guidelines. The following antibiotics were tested: penicillin, cefuroxime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, erythromycin, clarithromycin, azithromycin, roxithromycin, clindamycin, ciprofloxacin, levofloxacin, moxifloxacin, chloramphenicol, tetracycline, cotrimoxazole and vancomycin. Serotyping was performed by the capsular swelling method (Quellung reaction) with specific antisera available from the Statens Seruminstitute of Copenhagen, Denmark.
Results: Of the 417 isolates tested, 82 (19.7%) showed intermediate resistance and 69 (16.5%) high-level resistance to penicillin. Erythromycin, clindamycin, sparfloxacin, moxifloxacin, chloramphenicol, tetracycline, and cotrimoxazole resistance rates were 33.3, 11.5, 0.4, 0.2, 3.8, 27.3, and 30%, respectively. Multiple resistance was observed in 102 strains. All isolates were susceptible to ciprofloxacin, levofloxacin and vancomycin. Among the serotypeable strains, serotype 19F predominated, followed by serotypes 3, 6B, and 14.
Conclusion: The results of the present study indicate that continuous surveillance remains important for guiding empirical antibiotic treatment.

R2106 Ten-year survey of co-trimoxazole and quinolone resistance in *Escherichia coli* causing urinary tract infections

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Objectives: During the recent years, the treatment of choice for urinary tract infections in Greece has changed from co-trimoxazole to quinolones. Aim of this study was to determine the change in co-trimoxazole and quinolone resistance of *Escherichia coli* isolated from urinary tract infections during the last ten years in two groups, hospitalised patients and community patients.
Methods: Over a ten-year period (1999–2008) a total number of 4969 *Escherichia coli* clinical strains were isolated in our hospital laboratory from urine cultures of 3215 hospitalised patients and 1754 community patients with urinary tract infections. Classic microbiological techniques were performed for the urine cultures and identification of microorganisms. Antibiotic susceptibility was tested by the Kirby-Bauer method according to CLSI protocols.
Results: The co-trimoxazole resistance ratio increased gradually during this period from 13.4% and 21% for the community patients and hospitalised patients respectively in 1999 to 28.8% and 32.1% in 2008. The quinolone resistance for the community patients and hospitalised patients respectively, increased highly: from 2.8% and 7.3% in 1999 to 12.7% and 22.4% in 2008 for ciprofloxacin (2000: 4.7–7.9%, 2001: 2.8–8.9%, 2002: 3.8–12%, 2003: 6.1–13.4%, 2004: 7.5–15.7%, 2005: 6.2–14.9%, 2006: 4.6–17.6%, 2007: 6.2–19.7%) and from 2.9% and 7.1% in 1999 to 15.9% and 25.9% for norfloxacin (2000: 3–7.3%, 2001: 2.8–9.2%, 2002: 3.8–11%, 2003: 6.1–13.9%, 2004: 8.1–15.7%, 2005: 6.8–15.3%, 2006: 4.6–17.3%, 2007: 6.2–22.4%).
Conclusion: The co-trimoxazole and quinolone resistance of *Escherichia coli* in urinary tract infections was highly increased during the last ten years, not only in hospitalised but also in community patients. These findings emphasize that antibiotic usage polices, especially empirical therapies, should be based on antimicrobial resistance surveillance studies.

R2107 Comparison between hospital cumulative and specific antibiograms according to sampling time and type of unit

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Objectives: Empirical antibacterial therapy in hospitals is usually guided by local epidemiologic features reflected by institutional cumulative antibiograms. This study aimed at investigating the additional information inferred from specific antibiograms aggregated by sampling time, type of unit, or type of sample.
Methods: Antimicrobial susceptibility rates of different pathogens (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, enterococci) were collected over a four-year period in a tertiary care, university-affiliated hospital. Hospital-wide data were compared with those selected by type of unit (medical, surgical, paediatric, ICU), sampling time < or >48 hours after hospital admission (presumably reflecting a community or hospital origin, respectively) and type of sample (blood vs any other site).
Results: Strains isolated >48 h after hospital admission were significantly less susceptible than those presumably arising from the community (<48 h). The comparison of units revealed significant differences among strains isolated >48 h after admission. When compared to hospital-wide antibiograms, susceptibility rates were lower in ICU and surgical units for *E. coli* with respect to amoxicillin/clavulanate (75 and 70%, respectively, vs 81%, $p < 0.01$), and enteroccocci to penicillin (70 vs 86%, $p < 0.001$) and in medical units for *S. aureus* with respect to oxacillin (70 vs 84%, $p < 0.001$). Important differences were also observed between units for *P. aeruginosa* (Figure). In contrast, few significant differences between units were observed among strains isolated within the first 48 h from admission. Distinction according to the type of sample (blood vs any other site) also did not show relevant differences.

Conclusion: Important variations in antibacterial susceptibility were observed in a same hospital according to the time of sampling (< or >48 hours from admission) and type of unit. Hospital-wide antibiograms reflect the actual susceptibility pattern for a specific unit with respect to presumably community-acquired (<48 h), but not to hospital-acquired strains (>48 h). Antibiograms specifically adjusted to these parameters are easy to obtain and may be useful in guiding the choice of empirical antibacterial therapy.

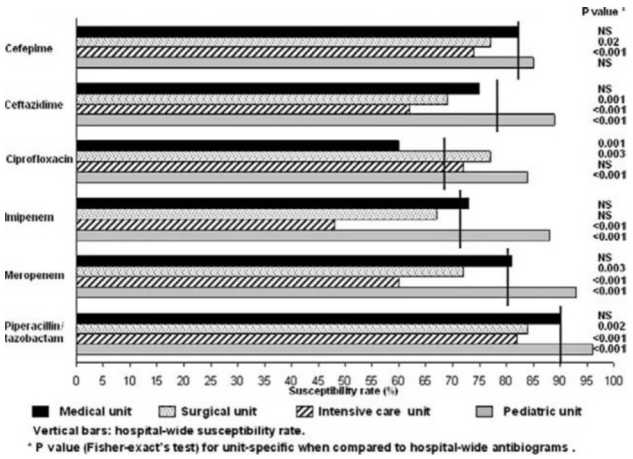

Figure: Susceptibility rate of *P. aeruginosa* strains isolated >48 h after admission.

R2108 Increasing prevalence of extended spectrum β-lactamases producers among common uropathogens in a Greek tertiary-care hospital: a 3-year comparative study

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Objectives: Urinary tract infections (UTI) remain the most frequent infections diagnosed in outpatients as well as in hospitalised patients. We comparatively evaluated the prevalence of extended spectrum β-bactamases (ESBL) producing (ESBL+) *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* among urinary isolates during the last three years. Resistance rates to fluoroquinolones (FQs) and trimethoprim/sulfamethoxazole (TMP-SXT), antimicrobial agents commonly prescribed as per os treatment in patients with UTI, were also studied.

Methods: A total of 3842 strains isolated from urine cultures were collected from 2685 hospitalised patients and 1157 outpatients during a 3-year period (2006–2008). Species identification and antimicrobial susceptibility testing was performed by VITEK 2 Compact automated system (bioMérieux). ESBL-production was screened by double-disc synergy test (DDST) and confirmed by combined disk test (ceftazidime and cefotaxime with and without clavulanate), according to CLSI guidelines.

Results: Among urinary isolates, the prevalence of ESBL-producers was 1.3% in 2006 (17/1300), rising to 2.2% in 2007 (29/1326). A remarkable increase (7.1%) in ESBL producers was noted in 2008: 86/1216 isolates carried ESBL. Regarding *E. coli*, the number of ESBL+ isolates rose from 12 in 2006 to 22 in 2007. A more than 5-fold increase was observed in 2008 (69 isolates). The increase of prevalence of ESBL producers was significant but less notable for *K. pneumoniae*: 5, 7 and 14 were recovered in 2006, 2007 and 2008 respectively. *P. mirabilis* ESBL+ was first detected in 2008 (3 isolates). The comparative study of resistance rates revealed that non-ESBL producers expressed low resistant rates to FQs and TMP-SXT as compared to ESBL producers. No significant differences were observed in resistance rates from 2006 to 2008. Resistance rates are presented in detail in the table.

Conclusion: Increasing spread of ESBL-producers among urinary isolates along with high resistance rate to fluoroquinolones and trimethoprim/sulfamethoxazole, either in hospital or community settings, generate a therapeutic challenge because of limited oral therapeutic options. Monitoring of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with UTI.

	% resistance					
	2006		2007		2008	
	FQs	TMP-SXT	FQs	TMP-SXT	FQs	TMP-SXT
ESBL–	12.6	28.9	16.4	21.8	14.4	25.7
ESBL+	88.2	82.3	86.2	68.9	82.0	66.0

R2109 Inadequate drugs for the treatment of Gram-negative infections based on the EPICENTER Network data

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Objectives: Surveillance data must help to identify drugs, which are obsolete for the treatment of infections because of resistance development. We analysed a one year period of the EPICENTER Network data to categorise drugs.

Methods: At present 4 laboratories participate in the network using the automated BD PHOENIX system measuring MIC's. The BD EPICENTER Data Management System is used for the evaluation of the data in the laboratory and for the transfer of the data to the concentrator for evaluation with stratification by material, source, medical discipline, time, patient and others. Copy strains are excluded. Quality control is mandatory. Antibiotics with more than 35% resistance for a species were regarded as obsolete for empirical treatment.

Results: We analysed 949 *Enterobacter cloacae*-, 5500 *E. coli*-, 613 *Klebsiella oxytoca*-, 1285 *Klebsiella pneumoniae*-, 759 *Proteus mirabilis*-, and 1459 *Pseudomonas aeruginosa* strains. Data are presented in the table. Blank fields indicate a drug–bug combination which is regarded as obsolete and was not tested or documented. The isolates were from all specimen types. Analysing the data by specimen types, urine, blood, or pulmonary tract isolates the stratification is slightly different, but the general outcome for those drug–bug combinations in question is similar. According to our data all tested aminoglycosides are still appropriate drugs. The same holds true for aztreonam, cefepime, ceftazidime, meropenem piperacillin/tazobactam, and surprisingly ciprofloxacin. The percentage of resistance to fosfomycin is still low, but it can only be used in combination with other antibiotics. Amoxicillin should not be used for empirical therapy for Gram-negative infections. Clavulanic acid in combination with amoxicillin does not improve the situation significantly. Also Tetracyclin and cotrimoxazole should be used with care.

Antimicrobial	Antibiotic resistance of Gram-negative isolates					
	E. cloacae	*E. coli*	*K. oxytoca*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Amikacin	0.10%	0.20%	0.20%	0.20%	0.00%	4.30%
Gentamicin	2.10%	7.20%	1.60%	5.10%	5.40%	14.50%
Tobramycin	2.40%	7.40%	1.50%	5.40%	6.90%	5.80%
Amoxicillin/Clavulanic acid	**R**	**39.10%**	26.70%	21.00%	14.20%	R
Ampicillin	**R**	**49.50%**	**R**	**R**	31.10%	**R**
Aztreonam	23.20%	6.30%	15.70%	7.50%	10.00%	33.70%
Cefazolin	**R**	11.80%	31.60%	14.00%	9.10%	R
Cefepime	2.90%	6.40%	15.80%	7.60%	1.50%	21.00%
Cefotaxime	26.60%	6.00%	15.70%	7.50%	1.10%	**R**
Ceftazidime	25.10%	6.10%	13.90%	7.50%	1.10%	19.50%
Cefuroxime	**49.00%**	10.00%	22.20%	17.90%	4.80%	**R**
Imipenem	0.70%	0.10%	0.30%	0.10%	**39.10%**	27.00%
Meropenem	0.40%	0.00%	0.20%	0.20%	0.00%	14.20%
Piperacillin	27.00%	**45.40%**	21.40%	19.50%	16.70%	21.20%
Piperacillin/Tazobactam	19.60%	4.10%	18.40%	9.30%	4.10%	18.70%
Trimethoprim/sulfamethoxazole	8.90%	31.40%	7.80%	14.30%	**56.00%**	**R**
Fosfomycin w/G6P	3.70%	0.10%	3.20%	3.20%	0.00%	0.00%
Ciprofloxacin	3.20%	19.80%	6.40%	8.80%	12.70%	16.70%
Levoflaxacin	3.40%	19.80%	5.20%	8.80%	13.40%	**35.50%**
Tetracycline	9.70%	**38.20%**	9.70%	19.60%	**R**	**R**

Conclusion: Based on resistance %ages stored in the EPICENTER Network amoxicillin, amoxicillin/clavulanate, tetracyclin and cotrimoxazole

should only be used in Germany to treat infections with Gram-negative bacteria if local or patient specific data are available that suggest that therapy will be successful in more than two thirds of the patients.

R2110 Emergence of metallo-β-lactamase-producing *Escherichia coli* in a neonatal intensive care unit

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Objectives: Isolation of metallo-β-lactamase (MBL)-producing Gram-negative bacteria represents a serious health care problem. Recently, MBLs have been also sporadically detected among *E. coli* isolates causing hospital-acquired infections in the adult population. However, there are not reports describing MBL-producing *E. coli* in neonates with or without prior antimicrobial exposure. We present a case of MBL-producing *E. coli* colonisation in a 12-day old neonate, admitted to a neonatal intensive care unit (NICU).
Methods: The neonate was admitted from home to the NICU with fever (38.5ºC) and failure to feed properly. The neonate was not treated with antibiotics and the mother had no exposure to antibiotics during pregnancy. Surveillance swabs were taken from the rectum upon admission and cultured in selective medium. The carbapenem-resistant isolate was identified with API 20E (Biomerieux, Marcy-L'Etoile, France) and antimicrobial susceptibility testing was determined using the disk diffusion method employing CLSI criteria. Imipenem and meropenem MICs were determined using Etest (AB Biodisk, Solna, Sweden). The Etest MBL (AB Biodisk) was used for phenotypic detection of MBL production. PCR and sequencing assays were used for MBL gene detection.
Results: A strain of *E. coli* was identified which exhibited resistance to all β-lactams and β-lactam/β-lactamase inhibitors, except aztreonam. The strain was also resistant to aminoglycosides, except gentamicin, and remained susceptible to ciprofloxacin. Imipenem and meropenem MICs exceeded 32 mg/L. Etest-MBL was positive, indicating production of MBL. PCR revealed production of a VIM-type MBL gene and sequence analysis identified blaVIM-1 gene in a class 1 integron structure. The neonate was discharged two days after the admission in good condition.
Conclusion: MBL-producing *E. coli* colonisation is described for the first time in the neonatal population. It is noteworthy that a prior exposure to carbapenems or other antimicrobials was not reported. Such a study may highlight the need for implementation of microbiological screening tests and strategies to prevent MBL-producing colonisers from becoming health care-associated infections in NICUs.

R2111 Emergence of increased mupirocin resistance in Scottish MRSA isolates

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Objectives: An increase in high level mupirocin resistance in meticillin resistant *Staphylococcus aureus* (MRSA) bacteraemic isolates has been observed in Scotland. Mupirocin was introduced into clinical practice in the United Kingdom in 1985. It is used routinely for the elimination of nasal carriage of MRSA and as an effective treatment of skin infections. Resistance to mupirocin was observed worldwide not long after its introduction however Scotland has recently witnessed a significant increase in the number of mupirocin resistant MRSA strains. This increase in resistance has significant clinical implications for the treatment of mupirocin resistant MRSA infection and colonisation.
This study will describe trends in mupirocin resistance in bacteraemic MRSA isolates. The results can be used as a proxy indicator of the overall MRSA mupirocin resistance for all isolates in Scotland.
Methods: Scotland participates in the European Antimicrobial Resistance Surveillance System (EARSS) and the reporting of *Staphylococcus aureus* bacteraemic isolates to this surveillance system is mandatory.
Validated data collected over a two year period will be analysed for the detection of significant changes in mupirocin resistant MRSA isolates.
Results: The graph illustrates the increase in the number of MRSA isolates resistant to mupirocin within the time period January 2007 to October 2008.
Initial analysis suggests that the proportion of mupirocin resistance was significantly higher in 2008 as compared to 2007 (OR = 3.48; 95% CI 2.01–6.21; $p < 0.00001$).
Conclusion: Preliminary analyses suggest that there has been a statistically significant increase in resistance to mupirocin in MRSA bacteraemic isolates reported to EARSS between January 2007 and October 2008.
Further work is required to investigate the clinical impact of the emergence of increased mupirocin resistance. Scotland is currently conducting pilot studies into the viability of universally screening patients for the presence of nasal MRSA upon admission. MRSA resistance to mupirocin would have an impact on the choice of antibiotic used for MRSA clearance as there are a limited number of antibiotics licensed for this purpose.

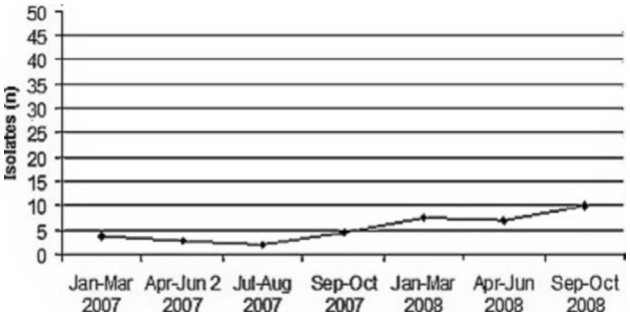

Figure 1. MRSA mupirocin bacteraemic isolates (2007 to 2008).

R2112 *Acinetobacter baumannii* resistance rates in a tertiary hospital

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A. baumannii is a well recognized pathogen causing a wide variety of infections, especially in ICU patients. Colonisation of patients from resistant strains usually precedes infections that are difficult to control by antimicrobial agents.
Objectives: To determine *A. baumannii* antibiotic resistance and tigecycline MIC distribution
Methods: 1120 *A. baumannii* strains were recovered in our laboratory during one year (January to December 2008). Specimens were obtained from ICU (93.8%), medical wards(2.9%), surgical wards 1.5% and outpatient clinic(1.8%). Clinical samples were: bronchial secretions 58.3%, rectal swabs 23.5%, wounds and pus 5.9%, catheter tips 3.8%, urine 3%, BAL 2%, sputum 1.7%, blood 1.4%, pleural and peritoneal fluid 0.4%. Antimicrobial susceptibility testing was performed for tazo+piperacillin and meropenem by automated system(Phoenix BD) and for colistin and tigecycline by E-test according to the manufacturer's instructions. CLSI interpretative criteria and guidelines were used.
Results: In 1120 strains resistance rates were: tazo+pip 92.8% resistant(R), 3.7% intermediate(I), meropenem 49.6% R, 31.7% I, ciprofloxacin 98.3% R, gentamicin 73% R, 2.1% I, trimethoprim-sulfamethoxazole 94.4% R. ICU isolates were not significantly more resistant than the rest of the isolates. Out of 414 strains 13% were resistant to colistin(MIC $\geqslant$ 4 mg/L) and MICs of 2, 1 and $\leqslant$0.5 mg/L were detected in 2.4%, 22% and 62.6% of strains respectively. Among 464 strains, MICs to tigecycline $\geqslant$6, 4, 3, 2 and $\leqslant$1 mg/L was detected in 25.4%, 50.9%, 17%, 5% and 1.7% respectively. Out of 167 meropenem resistant strains 22(13.2%) were also resistant to colistin. Sixteen of them were further tested for tigecycline MIC: 15 of them had MIC = 4 mg/L and one = 3 mg/L.
Conclusion: Colistin remains active to the majority of *A. baumannii* strains in our hospital. For those strains that were resistant to colistin, MIC to tigecycline was relatively high. Further studies are needed to establish the extend to which tigecycline is useful in colistin resistant strains.

Surveys of molecular epidemiology of resistance and resistance genes, strains or serotypes

R2113 Distribution of integrons and SGI 1 among antibiotic-resistant human isolates of *Salmonella typhimurium* phage types DT104 and U302

V. Majtán, T. Majtán, L. Majtánová (Bratislava, SK)*

Objectives: At present, *Salmonella Typhimurium* is the second most frequent type of *Salmonella* isolated from human samples in the Slovakia. The majority of these isolates correspond to multidrug resistant phage types DT104 and U302. The aim of this study was to investigate the presence of the genetic elements, the class 1 integrons as well as of SGI 1 in the resistant and MDR *S. Typhimurium* DT104 and U302 strains.
Methods: A total of 90 *S. Typhimurium* strains (phage type DT104=35, phage type U302=55) isolated from human sources during year 2007 were included in this study. The strains were tested for susceptibility to eleven antibiotics by the disk agar diffusion method on Mueller-Hinton agar plates. PCR for the detection of the class 1 integrons was carried out using the 5′CS and 3′CS primer pairs and the detection of the left junction of SGI 1 was carried out with the primer pairs U7–612 and LJ-R1.
Results: From thirty five DT104 strains the pentaresistant phenotype (ACSSuT) in 30 strains was found. This same multidrug resistance was also expressed by the 14 strains of U302 phagetype. The dominant occurrence of this resistance is closely linked with the presence of the SGI 1 as well as integrons. Indeed, all isolates of DT104 with the ACSSuT type resistance harboured the SGI 1, which carriers two integrons (1.0 kbp and 1.2 kbp) encodes multidrug resistance. Among 14 pentaresistant strains of phage type U302, 13 strains were positive for the presence of integrons but only 11 of them harboured the SGI 1. Sequencing of the integrons in these isolates identified aadA2 and blaPSE gene cassettes. The results showed that the majority of multidrug resistant U302 strains studied possessed the same antibiotic resistance genes as well as the SGI 1 as the multidrug resistant DT104 strains.
Conclusion: We analyzed the frequency of antibiotic resistance and contribution of the presence of both integrons and SGI 1 to this resistance. We tested *S. Typhimurium* human strains of DT104 and U302 phage types isolated during a single year from a relatively restricted geographic area. Nevertheles, the data of molecular analysis suggested a relationship between these dominant phage types (DT104 and U302) with regard to multidrug resistance phenotype, integron profile and SGI 1 structure.
This work was supported by Ministry of Health of the Slovak Republic under the project Molecular analysis of antibiotic resistance of nontyphoid salmonella serovars.

R2114 Ocurrence of blaCTX-M-15 gene in multidrug-resistant *Citrobacter freundii*

S. Ferreira, A. Paradela, J. Velez, E. Ramalheira, T. Walsh, S. Mendo (Aveiro, PT; Cardiff, UK)*

Objectives: Extended spectrum β-lactamases (ESBL) is one of the most important resistance mechanisms among Gram-negative bacteria. ESBL producing strains are emerging worldwide and need a careful surveillance. We investigated the prevalence of ESBL's, mainly CTX-M type, among ESBL positive *Citrobacter freundii* isolates.
Methods: Six ESBL positive *Citrobacter freundii* were identified by the automatic VITEK 2 system and Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Étoile, France). Resistance profile was also determined by disc diffusion methods. Presence of ESBL was confirmed by Etest (AB Biodisk) ESBL with Cefotaxime/Cefotaxime + Clavulanic acid and Ceftazidime/Ceftazidime + Clavulanic acid strips, according to manufacturer's instructions. PCR, nucleotide sequencing and sequence analysis was employed to detect β-lactamase encoding genes.
Results: Among the isolates studied, CIT1 strain showed a highly resistance profile to β-lactams (ampicillin/sulbactam, cefotaxime ceftazidime, cefepime, piperacillin and piperacillin/tazobactam), aminoglycosides (gentamicin and tobramycin) and fluoroquinolones (ciprofloxacin and norfloxacin); susceptibility was shown only to carbapenems. Nucleotide sequence of PCR amplicons revealed the presence of blaCTX-M-15 associated with the insertion sequence ISEcp1 upstream. blaOxa-30 and TEM-1 were also detected. Plasmid mediated CMY ampC was detected in all isolates.
Conclusion: The ESBL's of CTX-M type were the most prevalent. Different genetic environments associated with ISCR1 and ISEcp1 were detected.
CTX-M-15 found is plasmid mediated and, therefore, represents a dissemination problem, as these genes can be easily mobilised between strains or even species. The presence of a high variety of ESBL's in a single isolate highlights the importance of routine detection of ESBL producers.

R2115 Clones, antibiotypes and virulence genotypes of group C streptococci from bovine mastitis

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Objectives: To determine the clonality, resistance to macrolides, lincosamides and tetracycline, and virulence genotypes of *Streptococcus dysgalactiae* subsp. *dysgalactiae* (Group C *Streptococcus*, GCS), an important pathogen in bovine mastitis.
Methods: A total of 18 alpha-haemolytic streptococci were recovered from 304 bovine mastitis milk samples collected at Portuguese herds during 2002–03. These isolates were selected in blood agar media and identified as *S. dysgalactiae* subsp. *dysgalactiae* (GCS) using biochemical tests (API-20 STREP, BioMérieux; BBL Crystal Gram-Positive, Becton Dickinson; Slidex Strepto Kit, BioMérieux) and by sequencing of the 16S rDNA gene. All isolates were typed by pulsed field gel electrophoresis (PFGE) with computer-assisted DNA-band analysis (BioNumerics v. 4.0 software, Applied Maths). Antimicrobial resistance against macrolides (erythromycin-E), lincosamides (pirlimycin-PRL) and tetracycline-T, and the macrolide-lincosamide resistance phenotypes (M/cMLS/iMLS) were evaluated by disk diffusion. The antimicrobial resistance genes tet(M), tet(O), tet(W), tet(L), tet(Q), tet(K), tet(S), mef(A), erm(A) and erm(B) and the virulence phage-encoded genes of the human pathogen *Streptococcus pyogenes* (speA, speC, ssa, spd1, speK, speM, speL, speI and slaA) were searched for by PCR. Induction of phage lysates was obtained using 2 mg/L of mitomycin C (Sigma).
Results: Four PFGE clusters comprised 55.6% of the GCS and only one was herd specific. Isolates resistant to both E and T (22%) were of phenotypes iMLS or cMLS and genotypes erm(B)-tet(O) or erm(A)-tet(M). Part of the isolates (16.7%) was E susceptible and PRL resistant. Among all isolates, the following associations of virulence genes were observed: speC-spd1 (33%); speK-speM (33%); speL-speM (22%). Phage lysates were obtained from part of the putative lysogenic cultures.
Conclusion: These results suggest an environmental source rather than contagious origin of bovine GCS causing mastitis in Portuguese farms. A phenotype of susceptibility to macrolides and resistance to lincosamides-LSA (previously detected in *Streptococcus agalactiae* of human origin) was found in GCS bovine isolates. The finding of *S. pyogenes* phage-encoded virulence genes suggests that *S. pyogenes* prophages may play an important role in the transmission of important virulence genes among animal GCS.

In vitro antibacterial susceptibility and drug interaction studies

R2116 In vitro antibiotic susceptibility of anginosus group streptococci strains isolated from oral and respiratory tract infections

G. Bancescu, I. Nistor, A. Bancescu, M. Andrei, S. Barbuceanu, M.A. Topolniski (Bucharest, RO)*

Objectives: The streptococci of anginosus group belong to the normal flora of the oral cavity, upper respiratory tract, gastrointestinal tract

and urogenital tract. However, these streptococci are often involved in the aetiology of different pyogenic infections. The aim of the present study was to investigate the susceptibility of anginosus streptococci strains (isolated either in pure culture or in association with other microorganisms, especially anaerobic bacteria) from pus samples collected from Romanian patients with different oral and respiratory tract infections (mostly oral abscesses and sinusitis).

Methods: The identification of the *Streptococcus anginosus* group isolates at the species level was done using the Rapid ID 32 STREP system (Bio-Mérieux, France) and some additional biochemical tests. The investigation of the susceptibility of the isolates to: penicillin G, amoxicillin, erythromycin, clindamycin, chloramphenicol and tetracycline was performed by the Etest (AB Biodisk, Solna, Sweden). The phenotype of erythromycin resistance was detected using the double disk diffusion test.

Results: The anginosus group isolates belonged to: *S. anginosus* – which predominated (70% of the strains), *S. constellatus* and *S. intermedius* species. The MICs values varied between: 0.002–0.75 mg/l for penicillin G, 0.016–0.5 mg/l for amoxicillin, 0.016–4 mg/l for erythromycin, 0.016–0.047 mg/l for clindamycin, 0.016–4 mg/l for chloramphenicol and 0.047–256 mg/l for tetracycline. Reduced susceptibility to β-lactam antibiotics was found among the strains belonging to *S. constellatus* species. About 10% of the strains were resistant to erythromycin and only the M phenotype was established. Almost half of the total number of the isolates were resistant to tetracycline.

Conclusions: The results indicated that the susceptibility of the oral streptococci isolates of clinical importance should be periodically tested, mainly to the widely-used antibiotics. Clindamycin was fully active (as chloramphenicol) and might represent a therapeutical alternative for patients allergic to β-lactam antibiotics, while tetracycline is not recommended in infections in which oral streptococci are involved. Due to their role in human pathology, the anginosus group streptococci merit to be identified at species level in clinical laboratory.

R2117 Daptomycin susceptibility in methicillin-resistant *Staphylococcus aureus* strains isolated from paediatric patients

R. Bandettini, E. Castagnola (Genoa, IT)*

Objective: Daptomycin is a cyclic lipopeptide antibiotic that is rapidly bactericidal in vitro against a broad spectrum of Gram-positive bacteria. Its mechanism of action involves calcium-dependent binding to the bacterial plasma membrane and disruption of membrane function. Resistance is rare.

The aim of our study is the evaluation of daptomycin susceptibility in MRSA strains isolated in paediatric patients.

Methods: MRSA strains isolated from 74 paediatric patients, between 2006 and 2008, were studied. Of all this strains 10.8% were isolated from infected wounds, 10.8% from bacteraemia, 9.5% from blood catheters, 8.1% from burns, 2.7% from conjunctivitis and 48.6% from nasal carriers. None of this strains were vancomycin and linezolide resistant.

The minimum inhibitory concentration (MIC) of daptomycin were determined by using E test. Moreover we studied the eventual slime production using Congo red agar.

Result: All strains of staphylococci were susceptible to daptomycin and MICs values were between 0.09 and 0.5 microg/ml (susceptible $\leqslant$1 microg/ml). None of 74 MRSA strains were slime producers.

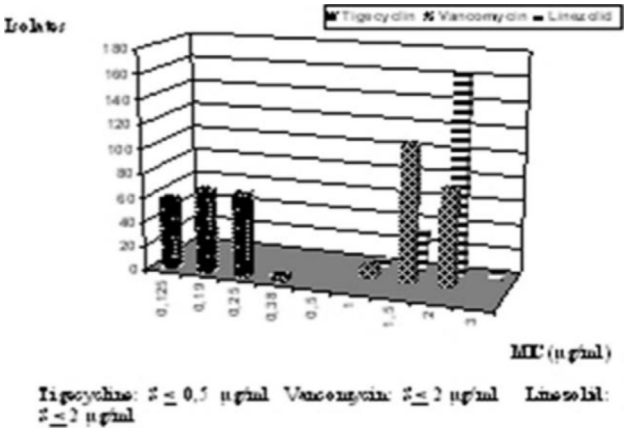
Conclusion: The increasing prevalence of Methicillin-Resistant *Staphylococcus aureus* requires the need to search for more effective agents. Vancomycin remains the reference standard for the treatment of systemic infection caused by MRSA. However the need for alternative therapies has become apparent, as a result of limited tissue distribution, as well as the emergence of isolates with reduced susceptibility to Vancomycin. Our study indicates, as data reported in literature, that daptomycin seems to be effective in vitro against MRSA. However further studies are needed to asses the pharmacokinetics, pharmacodynamics, safety and effectiveness of daptomycin in infants and children.

R2118 In vitro activity of tigecycline versus imipenem and versus vancomycin and linezolid against clinical isolates of ESBL-producing *E. coli*, *Klebsiella* spp. and methicillin-resistant *S. aureus*

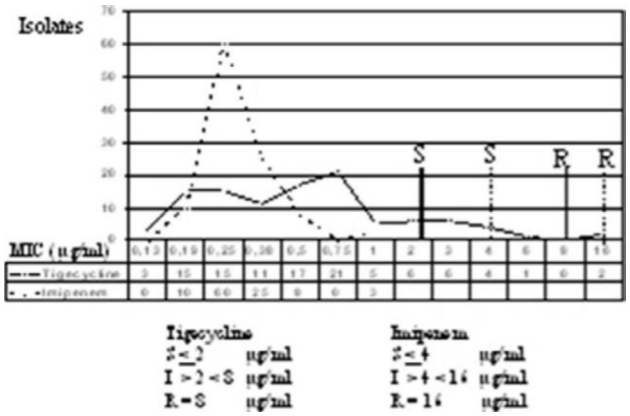
S. Scheithauer, H. Haefner, S. Lemmen (Aachen, DE)*

Objectives: Multiresistance in bacteria is an increasing problem in infection control and infectious diseases. Thus new therapeutic options are urgently needed. The aim of this study was to compare the local in vitro activity of Tigecycline with Imipenem against ESBL-producing Enterobacteriaceae and with Vancomycin and Linezolid against Methicillin resistant *S. aureus* (MRSA).

Methods: 306 clinical isolates (70 *E. coli*, ESBL+; 36 *Klebsiella* sp., ESBL+; 200 MRSA) were collected consecutively between 7/2005 and 3/2007 at the University Hospital Aachen, Germany. The Enterobacteriaceae strains were mainly isolated from urine (61%), blood culture (18%), and respiratory secretions (14%), the MRSA strains were mainly isolated from wounds (34%), blood culture (29%), and respiratory secretions (21%), respectively. For identification the Phoenix expert system (BD, Germany) was applied, MIC testing was performed by e-test. The breakpoints were in accordance with CLSI, USA.



MICs of tigecycline, vancomycin and linezolid against 200 MRSA strains.



MIC distribution of tigecycline and imipenem against *E. coli* and *Klebsiella* spp., ESBL-positive (n = 106).

Results: The distribution of the MIC values is shown in the figure. 67/70 (96%) *E. coli*, 26/36 (72%) *Klebsiella* sp. and all MRSA isolates were fully sensitive against Tigecycline. 3/70 (4%) *E. coli* and 8/36 (22%) *Klebsiella* sp. showed intermediate sensitivity against Tigecycilne, 2/36 (6%) *Klebsiella* sp. isolates were resistant. All ESBL-producing strains were tested sensitive against Imipenem, all MRSA isolates were tested

sensitive against Vancomycin and Linezolid. However in 79/200 (40%) a MIC of 2 μg/ml against Vancomycin and in 162/200 (81%) %) MIC of 2 μg/ml against Linezolid was detected.
Conclusion: The in vitro sensitivity of Tigecycline against ESBL-producing Enterobacteriaceae ranged between 72% for *Klebsiella* sp and 96% for *E. coli*. Tigecycline seems to be an effective alternative option for treatment of infections due to these bacteria, however antimicrobial resistance testing is recommended before starting therapy.
All MRSA strains were sensitive against the tested antibiotics, thus Tigecycline can be used for the indications studied and recommended.

R2119 Antimicrobial activity of seven chemotypes of *Lippia alba*

C.M. Oliveira Nunes, A.P. Delamare, F. Formolo, L. Michelim, S. Echeverrigaray (Caxias do Sul, BR)*

Objectives: *Lippia alba* is an American species of the Verbanaceae family currently used in Brazilian popular medicine with pharmacological antimicrobial, analgesic, and spasmolytic. The biological activity of this plant has been attributed to its essential oil. Several chemotypes of *Lippia alba* have been described. These chemotypes are characterised by high concentration of different mono and sesquiterpenes. Essential oils are considered natural non-toxic antimicrobial agents, and its efficiency depends on their chemical composition. In the present work we evaluate the antimicrobial activity of the essential oils of seven chemotypes of *Lippia alba* (linalool, limonene, citral, caryophyllene, camphor, carvone, and 1,8-cineole/camphor) against a panel of nine yeasts and 22 bacterial species.
Methods: Essential oils were obtained by hydrodistillation and their composition evaluated by GC and GC-MS. Minimal inhibitory concentration was determined by a microtiter plate method using serial oil dilutions (0 to 10 ml/L). Bacterial or yeast growth was evaluated in a microtiter plate reader at 592nm. The effect of oil concentration on biofilm formation was evaluated using safranine staining of bacterial cells attached to the polypropylene microtiter plates.
Results: The results obtained showed that the antimicrobial and biofilm inhibitory activities varied among chemotypes. The most efficient chemotypes were citral and caryophyllene inhibiting the highest number of bacterial species at the lowest oil concentration. The least efficiency was obtained with the carvone chemotype. Citral and caryophyllene oils were particularly efficient against *Bacillus* species (*B. megaterium*, *B. subtilis*, *B. cereus*), *Listeria monocytogenes*, *Enterococcus*, *Staphylococcus*, *Aeromonas*, and *Serratia*. In general, Gram-positive bacteria species were more sensitive than Gram-negative ones. All the chemotypes reduced biofilm formation, particularly those characterised by high concentration of oxygenated terpenes (linalool, citral and 1,8-cineole). Citral, carvone and limonene were the most efficient oils against yeasts, reducing both growth and biofilm.
Conclusion: These data showed that antimicrobial activity is highly dependent of essential oil composition, and this fact should be take in account for the proper therapeutic use of these and other natural products.

R2120 The investigation of the correlations between antibiotics and host immune effectors on virulence and antibiotic resistance of some *Escherichia coli* strains

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Objectives: The purpose of the present study was to investigate the influence of the combined action of antibiotics and different host immune effectors on the expression of antibiotic resistance and adherence capacity to the cellular and inert substrata in Escherichia (E.) coli strains.
Methods: This study was performed on two *E. coli* strains isolated from cardiovascular infections exhibiting resistance to the 3rd generation cephalosporins one by the production of the extended spectrum β-lactamase (ESBL) and the other by membrane impermeability mechanism, also, on the reference strains *E. coli* ATTC 25922. The capacity of adherence to the cellular substratum represented by HeLa cells (performed by Cravioto's adapted method) and, also, the adherence to inert substratum (quantified by rapid microtiter assay) were performed in the presence/absence of sub-inhibitory concentrations (SiC) of cephtazidime (CAZ) and nalidixic acid (NA) as well and/or different associations of host immune effectors (lyzosyme, complement C4, IgG, IgG+IgA+IgM) (in similar concentrations with those existing in the immunocompetent adult host).
Results: The tested strains presented different behaviours in their interaction with cellular and inert substrata in the presence of antibiotics and host immune effectors. In the case of *E. coli* ATCC 25922 strain the SiC of NA stimulated the capacity of adherence to cellular and inert substrata, but the SiC of NA and CAZ in association with the host immune effectors stimulated only the adherence to the cellular substratum, and inhibited the adherence to the inert substratum. For the ESBLs producing *E. coli* strain the SiC of NA, CAZ as well and the SiC of NA in association with opsonines induced the increase of adhesins synthesis. The SiC of CAZ in association with the host immune effectors significantly decreased the capacity of adherence to cellular substratum. The capacity of adherence to the inert substratum was not influenced by the SiC of antibiotics as well or in association with the host immune effectors. For the *E. coli* strain resistant by a membrane impermeability mechanism the SiC for both antibiotics stimulated the capacity of adherence to the cellular and inert substrata.
Conclusion: Our results prove that any immunological imbalance, characterised by the quantitative decrease or increase of one of the soluble host immune effectors, may favour, even in the antibiotic presence, the initiation of an infectious process.

R2121 Antimicrobial resistance of *Streptococcus pneumoniae* in a Vilnius university children's hospital, 2000–2008

I. Narkeviciute, G. Bernatoniene (Vilnius, LT)*

Objectives: *Streptococcus pneumoniae* (SP) is one of the most frequent causes of bacterial diseases in children. Local data on antimicrobial resistance is necessary in the empirical therapy. The aim of the study was to evaluate antimicrobial resistance of SP in 2000–2008.
Methods: SP strains were isolated from sterile sites (blood and CSF) and non-sterile sites (sputum, ear swab etc) from hospitalised children. Susceptibility of SP to penicillin and cefotaxime were performed using E-test (AB Biodisk, Sweden) and to erythomycin, clindamycin and vancomycin – using the disk diffusion method according to the CLSI criteria.
Results: Overall rates of resistance to antimicrobial agents in SP are given in the Table.

Antimicrobial agent	*S. pneumoniae* resistance (%)	
	Sterile sites	Non-sterile sites
Penicillin	2.4	4.6
Erythromycin	12.5	10.3
Clindamycin	7.3	4.7
Cefotaxime	0	1.9
Vancomycin	0	0.4

Conclusions: Highest rate of resistance has been observed for erythromycin in SP. Resistance to penicillin is low at present and resistance range was lowest against isolates from blood and CSF. SP strains appeared to have stable sensitivities to cefotaxime and vancomycin.

R2122 Antibiogram profile of potential probiotic *Bifidobacterium* spp. recovered from faeces sample of human origin

M. Reyed (Alexandria, EG)*

In the present study, a total of Sixty one strains belonging to potential probiotic Gram-positive, nonsporeforming, anaerobic bifidobacterial

strains isolated from human faecal samples were evaluated with regard to the antibiotic resistance. All strains were identified as *B. longum*, *B. breve* and *B. adolescentis*.

Spectrum of antibiotics included commonly used medicaments in human medicine. The antibacterial sensitivity profiles were studied by a microdilution broth method. Probiotic lyophilised stock culture *Bifidobacterium bifidum* 791 "Bifidumbacterium" from culture collection was taken as a control. Most strains of all types resisted 100 μg/ml or more of Kanamycin, Ofloxacin, Ciprofloxacin, Tetracycline, Gentamycin and Erythromycin.

8 Bifidobcaterial strains with high resistance to antibiotics were selected. The effect of UV on antibiotic and susceptibility was examined for 3 potent antibiotic resistance strains of bifidobacteria.

They were shown that the resistance to Ciprofloxacin, Cefazolin and Amoxicillin. Antibiotics resistant Bifidobacteria interact effectively and adhesive with mucous membrane of mouse in vitro and survive successfully in gastrointestinal tract of mouse in presence of antibiotics. These data are discussed in relation to the effect of antimicrobial agents on bifidobacteria in the normal human faecal microbiota, in relation to the implications.

In mouse oral administration by Ciprofloxacin (5 mg/kg body weight) are sufficiently high disrupt the micro-ecological balance of the microbiota in the bowel. The total number of lactobacilli and Bifidobacteria were all reduced in number and Enterobacteria, Enterococci and Staphylococci reach high numbers.

Resistant Bifidobacterial strains can effectively suppress excessive multiplication of opportunistic microorganisms in mouse intestines, caused by Ciprofloxacin induced dysbacteriosis and reestablish normal level of lactobacilli and Bifidobacteria normal microbiota population of the gut

These results demonstrate that the screening procedures developed in this study are effective for the selection of resistant *Bifidobacterium* strains, and developed criteria for in vitro selection of probiotic bacteria that may reflect certain in vivo effects on the host such as modulation of gastrointestinal tract microbiota.

New antimicrobials

R2123 In vitro activity of tigecycline and other broad spectrum antibiotics against micro-organisms isolated from infected patients in Colombia

A.L. Leal, G. Buitrago, J.A. Cortes, M.V. Ovalle, C.A. Alvarez, J.S. Castillo, J. La Rotta, S. Galo on behalf of the Colombian Group For Tigecycline Susceptibility Surveillance*

Objective: There are usually changes in the susceptibility profiles of antibiotics after their introduction in clinical use. Surveillance studies are important because they show the magnitude and trend of the change. A surveillance of tigecycline, two years after its introduction in Colombia is presented.

Methods: A surveillance study from 13 institutions in 8 different cities in Colombia was made. Microorganisms isolated from hospitalised patients with a microbiologically proven diagnosis of infection were sent to a reference centre. Antimicrobial activity was tested by a microdilution method for tigecycline (Trek diagnostics, London) and other broad spectrum antibiotics (MicroScan, Dade Behring, CA) commonly used. For interpretation CLSI rules were used and, for tigecycline, FDA approved breakpoints were used. No *Pseudomonas* species were collected.

Results: 802 isolates were collected (*S. aureus* 28%, *E. coli* 26%, *Klebsiella* spp. 18%, *A. baumannii* 10%, *Enterococcus* spp.7%, *Enterobacter* spp. 6% and *S. marcescens* 5%). Isolates were recovered from bloodstream (33%), skin and soft tissue (17%), surgical site infection (17%), abdomen (15%), respiratory tract (14%) and bone (4%). Susceptibility is shown according to the infection site and microorganism in Table 1.

Conclusion: Susceptibility profile of tigecycline is still very high after 2 years of clinical use. A high resistance profile is observed among collected isolates and limited therapeutic options are available.

Table 1. Susceptibility according to the infection site and microorganism

	n	% S											% S				TGC		
		OXA	VAN	AMK	FEP	CIP	CAZ	CRO	IMI	MEM	TZP	% ESBL	SAM	CSL[a]	COL[a]	MIN[a]	%S	MIC$_{50}$	MIC$_{90}$
Blood	**265**																		
Acinetobacter baumannii[b]	22			36	31	31			31	31	27		50	0	90	86	100	0.5	1
Enterobacter spp.	15			86	80	86	66	66	100	100	73		13				100	0.5	1
Escherichia coli	72			98	79	68	79	79	100	100	87	16.7	41				100	0.25	0.5
Klebsiella spp.	56			87	71	76	71	71	92[c]	87[c]	71	32.1	55				100	0.5	1
Serratia marcescens	13			53	76	100	100	61	100	100	92		0				100	1	1
Staphylococcus aureus	73	65	100			79											100	0.125	0.25
Enterococcus spp.	14		93														100	0.125	0.25
Skin and soft tissue	**137**																		
Acinetobacter baumannii[b]	9			11	11	11			22	22	11		33	0	100	100	100	0.25	1
Enterobacter spp.	11			72	81	54	72	63	100	100	81		9				100	0.5	1
Escherichia coli	22			86	63	54	63	63	100	100	86	31.8	31				100	0.25	1
Klebsiella spp.	15			93	80	73	80	80	93[c]	93[c]	60	20	46				100	0.5	1
Serratia marcescens	11			72	90	90	81	81	100	100	81		9				100	0.5	1
Staphylococcus aureus	61	62	100			90											100	0.125	0.25
Enterococcus spp.	8		100														100	0.125	0.25
Abdominal	**120**																		
Acinetobacter baumannii[b]	10			40	0	0			10	10	0		10	0	90	80	100	0.5	1
Enterobacter spp.	8			75	62	75	50	50	100	100	50		25				100	0.5	1
Escherichia coli	58			98	86	70	86	86	100	100	79	10.3	29				100	0.25	0.5
Klebsiella spp.	21			85	66	76	71	71	100	100	42	28.6	42				100	0.5	1
Serratia marcescens	2			100	100	100	100	100	100	100	100		0				100	0.125	0.5
Staphylococcus aureus	8	25	100			37											100	0.125	0.25
Enterococcus spp.	13		100														100	0.125	0.25
Respiratory tract	**111**																		
Acinetobacter baumannii[b]	21			33	4	4			9	9	9		9	0	76	100	100	0.5	1
Enterobacter spp.	5			100	60	80	60	60	100	100	80		40				100	0.5	1
Escherichia coli	9			100	77	66	77	88	100	100	77	22.2	11				100	0.25	1
Klebsiella spp.	36			97	75	86	69	75	97[c]	97[c]	61	25	38				100	0.5	1
Serratia marcescens	8			100	100	87	100	100	100	100	100		12				100	0.5	2
Staphylococcus aureus	32	59	100			68											100	0.125	0.25
Enterococcus spp.	0																		
Surgical site infection	**136**																		
Acinetobacter baumannii[b]	12			66	66	58			75	66	66		75	0	91	83	100	0.25	0.5
Enterobacter spp.	11			54	36	54	27	36	100	100	36		18				100	0.5	1
Escherichia coli	43			81	79	69	79	79	100	100	74	14	27				100	0.25	0.5
Klebsiella spp.	12			75	58	50	58	58	100	91[c]	41	41.7	33				100	0.5	1
Serratia marcescens	4			75	100	25	75	75	100	100	100		0				100	0.5	1
Staphylococcus aureus	41	43	100			70											100	0.125	0.25
Enterococcus spp.	13		100														100	0.125	0.25
Bone	**32**																		
Acinetobacter baumannii[b]	6			16	16	0			33	33	16		16	0	83	83	100	0.25	1
Enterobacter spp.	2			50	50	50	50	50	100	100	50		50				100	0.25	1
Escherichia coli	4			100	100	75	100	100	100	100	100	0	50				100	0.25	1
Klebsiella spp.	3			100	33	66	33	33	100	100	33	66.7	33				100	1	1
Serratia marcescens	1			100	100	100	100	100	100	100	100		0				100	1	1
Staphylococcus aureus	13	53	100			84											100	0.125	0.25
Enterococcus spp.	3		100														100	0.064	0.125

[a] E-test susceptibility. [b] Enterobacteriaceae breakpoints for tigecycline. [c] Molecular confirmation.
OXA, oxacillin; VAN, vancomycin; AMK, amikacin; FEP, cefepime; CIP, ciprofloxacin; CAZ, ceftazidime; CRO, ceftriaxone; ESBL, extended-spectrum beta-lactamase; IMI, imipenem; MEM, meropenem; TZP, piperacillin/tazobactam; SAM, ampicillin/sulbactam; CSL, cefoperazone/sulbactam; COL, colistin; MIN, minocycline; TGC, tigecycline; %S, percent susceptibility.

R2124 Anti-picornaviral activity of novel pirodavir derivatives

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Objectives: The early steps of the Picornavirus replicative cycle seem to be priviledged targets for some antiviral compounds like Disoxaril and Pirodavir. These drugs were shown to be strong inhibitors of virus adsorption in Picornavirus infected HeLa cells. On the basis of drug sensitivity, the division of Rhinovirus in groups A and B was proposed. Pirodavir was shown to be effective on both several group A and B strains of Rhinoviruses. The main weakness of Pirodavir is its cytotoxicity on cell cultures at relatively low doses. In this work we tested some original Pirodavir derivatives, in order to find less toxic compounds with an improved protection index (PI).
Methods: Compounds I-002, I-230, I-232, I-273, I-373, I-473; I-501; I-502, I-602, I-702 were synthesized and purchased from IFI SPA (Rome). Rhinoviruses HRV14 (group A) and HRV39 (group B) were grown in HeLa cells (Ohio strain). Poliovirus 1, Echovirus 6, 9, 11, were propagated in HEp-2 cell culture. Coxsackievirus B1 and A9 were propagated in RD cell culture. The antiviral activity assay was carried out by the 50% plaque reduction assay.
Results: The substitution of the oxygen atom in the central chain of Pirodavir with an amino group resulted in an increased antiviral activity against Echo and Coxsackie viruses, although a decreased activity against Rhinoviruses and Poliovirus was observed. Compound I-232 was about ten times less toxic than Pirodavir, but it was equally active. The substitution of the oxygen atom in the central chain with a thio group (as in compounds I-373 and I-501) did not influence antiviral activity. The presence of a double oxygen group in the central chain (as in compound I-602) resulted in a lower cell toxicity and in a higher anti-rhinovirus activity. In fact, this compound had a PI > 700 against HRV14 while Pirodavir had a PI=250.
Conclusion: Our results indicate that new derivatives endowed with lower cytotoxicity and that a higher PI against some Picornaviruses can be obtained starting from the Pirodavir.

R2125 In vitro resistance development to tobramycin by *Pseudomonas aeruginosa* is minimised by addition of PTZ601

D. Daigle, J. Hamrick, M. Corrigan, D. Pevear, C. Burns, L. Xerri (Malvern, US)*

Objectives: Evaluate the potential for antagonism between tobramycin and PTZ601 (previously known as PZ-601 and SMP-601), an injectable broad-spectrum carbapenem antibiotic with activity against multidrug-resistant Gram-positive and Gram-negative bacteria, and study the effect of a tobramycin-PTZ601 combination on the propensity for resistance development by *Pseudomonas*.
Methods: Serial passages of the *P. aeruginosa* strain PAO1, possessing multiple resistance mechanisms, were performed daily for 20 days in Mueller-Hinton broth in the presence of sub-inhibitory concentrations of meropenem, PTZ601, tobramycin and of combinations of each of the carbapenems with tobramycin (0.5×MIC drug A plus 0.5×MIC drug B). Resistance mechanisms included inducible AmpC β-lactamase, resistance-nodulation-cell division efflux systems, and OprD down-regulation. The inoculum for each subsequent passage was taken from the first well demonstrating evident turbidity, according to the CLSI microdilution method. Checkerboard analyses were performed to define a lack of antagonism between PTZ601 and tobramycin.
Results: Following 20 passages of *P. aeruginosa* PAO1 in sub-inhibitory concentrations of individual agents (meropenem, PTZ601, and tobramycin), higher levels were required to achieve inhibition with each single antibiotic, increasing 128-fold for meropenem (0.5 to 64 mcg/mL), 16-fold for PTZ601 (4 to 64 mcg/mL), and 64-fold for tobramycin (0.5 to 32 mcg/mL).
Conclusions: This study shows that even though PTZ601 is only marginally active against *P. aeruginosa* PAO1 on its own (MIC of 8 mcg/mL), when it is combined with tobramycin, resistance development is minimised. The lack of in vitro antagonism between PTZ601 and tobramycin indicates that this empirical combination to provide adequate anti-pseudomonal coverage warrants further study.

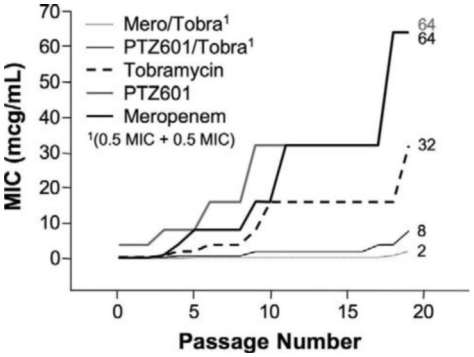

Figure: Effect of the combination of two carbapenems with tobramycin (0.5×MIC plus 0.5×MIC) on the development of resistance in serial passages of *P. aeruginosa* PAO1.

R2126 PTZ601: evaluation of the pharmacokinetics following single intravenous administration with and without cilastatin to male Sprague-Dawley rats and beagle dogs

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Objective: PTZ601 (previously known as PZ-601 and SMP-601) is a novel investigational parenteral 1β-methylcarbapenem active against a wide range of Gram-positive and Gram-negative bacteria, including multi-resistant pathogens. The objective of this study was to investigate the pharmacokinetics of PTZ601 in Sprague-Dawley rats and in beagle dogs after a single intravenous (IV) administration with or without cilastatin (a specific inhibitor of the renal enzyme dehydropeptidase I, responsible for the hydrolysis of many carbapenems).
Methods: PTZ601 (100 mg/kg) alone or in combination with cilastatin (100 mg/kg) was given by 10-minute IV infusion to 3 male rats or to 3 male dogs housed singly in metabolic cages for concomitant urine collection.
Results: In rats, the systemic clearance (CL) of PTZ601 administered alone was high (42.8 mL/min/kg), accounting for 62% of the hepatic blood flow (HBF). Renal clearance (CLr), 2.7 mL/min/kg, showed a limited contribution to total CL. Coadministration with cilastatin increased by 10 times the systemic exposure (AUC0-inf: 401.1 vs 43.3 ugxh/mL without cilastatin), with a concomitant marked reduction of CL (from 42.8 to 4.3 mL/min/kg), but a marginal effect on CLr (from 2.7 to 1.5 mL/min/kg). In dogs, PTZ601 CL without cilastatin (4.2 mL/min/kg) was found to be lower than that observed in rats and accounted only for about 10% of HBF. The CLr contribution (0.33 mL/min/kg) was proportionally similar to that observed in rats. In dogs, coadministration with cilastatin caused only a 2-fold increase of AUC0-inf (from 353 to 833 ugxh/mL) with a limited effect on both CL (4.2 vs 2.3 mL/min/kg) and renal elimination (CLr: 0.33 vs 0.57 mL/min/kg).
Conclusion: In rats, the coadministration of PTZ601 with cilastatin significantly reduced total clearance from 42.8 to 4.3 mL/min/kg. In dogs, the effect was much less pronounced. On the basis of these results, the subsequent nonclinical safety studies in rats, but not in dogs, were performed with cilastatin to increase exposure of PTZ601.

R2127 Antibacterial activities of cobweb protein

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Objectives: Cobweb production is an essential activity of spiders to capture their prey and to get nutrients from them. Spider web contains

various amino acids mainly glycine and alanine along with pyrrolidin, that retains water and prevents the web from drying up; potassium hydrogen phosphate and potassium nitrate, which are known to prevent fungal and bacterial growth on the web. The objective of our experiment is to study the antibacterial activities of cobweb proteins on different bacteria.

Methods: Webs of common household spider – *Crossopriza lyoni* were collected with a clean glass rod and washed with deionised distilled water and dried in incubator. The spider silk then dissolved in hydrochloric acid and acetic acid (50:50 v:v) and neutralised with sodium hydroxide. After this proteins were separated and a solution of 1 mg/mL of the extracted protein was used for study of its antibacterial activities on international strains of *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and many wild strains of bacteria including MRSA, MBL-positive Gram-negative bacteria. The MIC values were determined after serial dilution of the protein in liquid M H broth in micro dilution plates followed by challenge with different microbes and measuring absorbances at 620 nm in Micronaut system (Germany).

Results: MIC values of all Gram negative organisms including MBL producing strains were much lower (<10 mcg/mL) than that of the Gram positive organisms (>30 mcg/mL).

Conclusion: Cob web protein showed prominent antibacterial activities. The difference in the activities of spider web proteins between Gram positive and Gram negative bacteria was well marked. Crude extract of spider web is included in pharmacopoeia of alternative medicines for psychological illnesses, thus it has got no toxic effect in human beings.

R2128 Efficacy of carbohydrate-derived fulvic acid against MRSA and *Candida albicans* in murine models of sepsis

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Objectives: Humic acid is a major component of humic substances which are dark brown constituents of soil organic matter formed by degradation of plants & animals in the environment. The fraction of humic acid that is soluble in water at all pH is fulvic acid but due to its colloidal nature is contaminated by organics and heavy metals. Carbohydrate Derived Fulvic Acid (CHD-FA) avoids contamination by production in industrial bioreactors from plant material free of contaminants. Here we investigated the antimicrobial efficacy of CHD-FA against MRSA and *C. albicans* in murine models of sepsis.

Methods: CHD-FA (Fulhold Ltd) naturally exists as a light brown substance pH 2.1 was buffered with NaOH to pH 5.0 for use. Male CD1 mice 22–25 g were used. In the *C. albicans* model (fluconazole resistant strain) mice were compromised with 1 dose of cyclophosphamide. In both models mice were infected IV with sufficient organisms to cause 100% mortality in untreated mice 5–7 days post infection.

Mice infected with MRSA were treated with 40 or 160 mg/kg CHD-FA BD oral, 80 mg/kg oxacillin (OXA) BD, CHD-FA + OXA, 50 mg/kg vancomycin (VAN) BD or vehicle. Treatment started 1 hour post infection.

Mice infected with *C. albicans* were treated with 25 or 100 mg/kg CHD-FA BD oral, 10 mg/kg fluconazole (FLU) once daily, CHD-FA + FLU, 0.5 mg/kg amphotericin (AMB) or vehicle 5 hours post infection. All animals were euthanised 50 h (MRSA) or 53 h (*Candida*) post infection. In both models the kidneys were quantitatively cultured to determine burden.

Results: CHD-FA at pH 5.0 and given orally was well tolerated. In the MRSA model vehicle treated mice had high burdens (~10^6 cfu/gm). Treatment with CHD-FA or OXA monotherapy had a modest effect on burden (~10^5 cfu/gm). In contrast 160 mg/kg CHD-FA + 80 mg/kg OXA was highly effective at reducing burden (<10^3 cfu/gm) $p = 0.01$ and equivalent to VAN $p > 0.05$. *C. albicans* infected, vehicle treated mice had high burdens (3.7×10^6 cfu/gm). Monotherapy with CHD-FA had a modest effect reducing burdens to ~1×10^6 cfu/gm; FLU monotherapy reduced the burden to ~4×10^4 cfu/gm. The combination of CFH-FA + FLU was superior to either monotherapy reducing the burden to ~1×10^4 cfu/gm ($p < 0.0001$ compared to vehicle) and equivalent to AMB ($p = 0.11$)

Conclusions: CHD-FA oral treatment was well tolerated in murine models of sepsis. Combination treatment of MRSA with CHD-FA + OXA and *C. albicans* treated with CHD-FA + FLU were highly effective at reducing the kidney burden.

R2129 Antibacterial activity of the polymeric guanidines in hygienic handwash against *Escherichia coli* K12 NCTC 10538

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Objectives: The cationic biocides Akacid® (Poly-[2-(2-ethoxy)-ethoxyethyl-guanidinium-chlorid]) and Akacid® Plus, a 3:1-mixture of Poly-(hexamethylen-guanidinium-chlorid) and Poly-[2-(2-ethoxy)-ethoxyethyl-guanidinium-chlorid], show broad in vitro activity against bacteria and fungi. The aim of this pilotstudy was to evaluate the activity of the novel polymeric guanidines against the non-pathogenic reference strain *Escherichia coli* K12 NCTC 10538 in hygienic handwash according to the test methods of the European Standard EN 1499.

Methods: Akacid® and Akacid® Plus at a concentration of 0.5 and 1% were tested compared to kalisoap in twelve different healthy volunteers. For artificial contamination the proband's hands were dipped into one litre of a bacterial suspension of *E. coli* K12 NCTC 10538 (2×10^8 to 2×10^9 cfu/ml) and were air-dried. To test the activity of Akacid® and Akacid® Plus, the test substances or the reference soap were applied and were rubbed either once or twice for one minute. The bacterial prevalues (after contamination) and postvalues (after disinfection and neutralisation) were determined according to the guidelines of the reference wash method EN 1499. In each case the reduction factor between prevalue and postvalue was calculated.

Results: In all test groups the prevalues of the bacterial counts ranged from 1×10^6 to 3×10^7 cfu/ml with a mean value of 1.3×10^7 cfu/ml. After single and double application of kalisoap alone mean reductions factors of 9.8×10^2 cfu/ml and 5.9×10^3 cfu/ml were determined. A significantly higher reduction of the microbial count was achieved after treatment with Akacid® Plus 1% as single and double application (mean reduction value 6.6×10^3 cfu/ml and 3.7×10^4 cfu/ml, respectively $p < 0.001$), whereas reduction values due to Akacid® Plus 0.5%, Akacid® 1% and Akacid® 0.5% were comparable to kalisoap.

Conclusion: We proved superiority of the new disinfectant solution Akacid® Plus 1% in reducing colony counts of *E. coli* K12 on hands of healthy volunteers compared to the reference substance kalisoap in hygienic handwash.

R2130 In vitro bactericidal and antibiofilm activity of six antimicrobial peptides against multidrug-resistant pathogens from patients with cystic fibrosis

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Respiratory tract infection is the major cause of morbidity and mortality in cystic fibrosis (CF). Physicians are increasingly faced with infection with multidrug-resistant isolates due to many prolonged courses of antimicrobial agents used to slow the rate of decline in pulmonary function. For this reason, new therapeutic approaches are needed to improve the management of CF lung disease, including development of novel agents to treat antibiotic-resistant pathogens.

Objective: To test in vitro the bactericidal activity and the effect on biofilm formation of six cathelicidin-derived antimicrobial peptides against eleven Gram-positive and -negative clinical isolates from CF patients. The peptides included members of the alfa-helical group (LL-37, SMAP-29, BMAP-27, and BMAP-28), the Trp-rich peptide indolicidin, and the Pro-rich peptide Bac7(1–35).

Methods: The bactericidal activity of the peptides was tested against multiply antibiotic-resistant strains of *Stenotrophomonas maltophilia* (n = 3), *Pseudomonas aeruginosa* (n = 4), and *Staphylococcus aureus* (n = 4), by using the broth microdilution assay according to the CLSI

guidelines. The effect of subinhibitory concentrations of peptides on biofilm formation was analyzed by crystal violet staining in polystyrene 96-well microtiter plates after 24 h of incubation at 37ºC. All assays were performed at least in duplicated and repeated twice.
Results: SMAP-29 was the most active peptide (MIC range: 2–32 microg/ml; MIC90: 8 microg/ml), followed by BMAP-28 (MIC range: 4–⩾64 microg/ml; MIC90: 16 microg/ml), and BMAP-27 (MIC range: 2–⩾64; MIC90: ⩾64 microg/ml). In contrast, indolicidin, LL-37, and Bac7(1–35) had no activity against the clinical isolates tested (MIC ⩾64 microg/ml). SMAP-29 and BMAP-27 were particularly active against *P. aeruginosa* (MIC range: 2–4 microg/ml) and BMAP-28 against *S. aureus* (MIC range: 4–8 microg/ml). These three peptides at 1/2×MIC significantly ($P < 0.05$) reduced biofilm formation by *S. maltophilia* and *P. aeruginosa* strains (biofilm reduction rate: from 26 to 93%, and from 28 to 83% vs control, respectively). SMAP-29 at 1/2×MIC was the only peptide that significantly ($P < 0.05$) decreased biofilm formation by *S. aureus* (biofilm reduction rate: from 70 to 72% vs control). In contrast, subinhibitory concentrations of indolicidin, LL-37, and Bac7 had no effect on biofilm formation.
Conclusion: SMAP-29, BMAP-27, and BMAP-28 display potential for development as therapeutic agents for CF lung disease.

R2131 Outcomes of pan-resistant *Acinetobacter baumannii* infections treated with tigecycline

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Objectives: To determine clinical and microbiological outcomes of patients treated with tigecycline for pan-resistant *Acinetobacter baumannii* infections.
Methods: The study was conducted at the Turkiye Yuksek Ihtisas Education and Research Hospital, a 419 bed, tertiary care hospital. All adult patients who received tigecycline at least 5 days for treatment of an infection due to pan-resistant *A. baumannii* included in the study and followed up prospectively. Tigecycline susceptibility was determined using the Etest method. *A. baumannii* strains were susceptible only colistin and tigecycline.
Results: Tigecycline therapy was administered to thirteen patients for pan-resistant *A. baumannii* infection. Of these, 11 patients who received tigecycline at least 5 days included in the study. Mean age of the patients was 64.3±10.6 (range, 40–79) and 8 (72.7%) was male. All of the patients had comorbidities, 6 (54.6%) had coronary artery disease, 6 (54.6%) had hypertension, 4 (36.4%) had diabetes mellitus and 2 (18.2%) had chronic obstructive pulmonary disease. All of the patients were followed up in ICUs. The mean time between hospital admission to the development of pan-resistant *A. baumannii* infection was 44.3±24.4 days. Ten of the patients had surgical operation. Seven (63.6%) patients had mechanical ventilation support. Seven (63.6%) patients had surgical site infection, 2 (18.2%) patients had ventilator associated pneumonia, one patient had mediastinitis and one patient had soft tissue infection due to pan-resistant *A. baumannii*. The mean duration of the tigecycline treatment was 12.4±5.4 (range 5–21) days. Five patients had additional nosocomial infections. In two patients imipenem was administered for additional infections further with tigecycline. Three patients had a fatal outcome and two deaths were thought to be related to the *A. baumannii* infection. The mean interval from the initiation of tigecycline treatment to death was 7.6 days. The mean APACHE II score was 9.4 in surviving patients and 17.7 for those who did not survive ($p < 0.001$). All of the patients with fatal outcome had surgical site infection. No microbiological failure was observed during the tigecycline treatment.
Conclusions: Tigecycline has the potential to be an option for pan-resistant *A. baumannii* infections including surgical site infection, ventilator associated pneumonia and mediastinitis.

R2132 In vitro activity of tigecycline alone and in combination with colistin methanesulfonate against carbapenem resistant *Acinetobacter baumannii* isolates

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Objective: *Acinetobacter baumannii* is a Gram-negative organism that has emerged as increasingly important nosocomial pathogens because of the extent of its antimicrobial resistance and its persistence in the hospital environment. Since it has an extraordinary ability to acquire antibiotic resistance, a large number of *A. baumannii* strains have been recently reported to be carbapenem resistant in many countries. Tigecycline, the 9-tert-butyl-glycylamido derivative of minocycline, is the first commercially available member of the glycylcyclines. Tigecycline has in vitro activity against *Acinetobacter* spp. and has been suggested as a therapeutic option in these infections. For these reasons, the aim of the present study is to determine the in vitro activities of tigecycline alone and in combination with colistin methanesulfonate against carbapenem resistant *A. baumannii* strains.
Methods: 50 clinical isolates were collected and identified in the Clinical Microbiology Laboratories of Istanbul University, Istanbul Faculty of Medicine. *Escherichia coli* ATCC 25922 was used as a control strain. Tests for the susceptibility to meropenem and tigecycline were performed by the broth microdilution method as recommended by CLSI guidelines. The MICs of colistin methanesulfonate were determined by using a microbroth dilution assay modified from the method of the CLSI. The effects of antibiotics in combination were assessed by using the microbroth checkerboard technique. With this method, synergy was defined as an FIC index of ⩽0.5, additivity as an FIC index of 1.0 and antagonism as an FIC index of ⩾2.0.
Results: The MIC values of the three antibiotics were ranked as follows: meropenem > colistin methanesulfonate > tigecycline. Tigecycline demonstrated excellent inhibitory activity against *A. baumannii* with MIC (90) ⩽0.5 mg/l. On the other hand, 32% of the strains were resistant to meropenem. According to our results, with a FIC index of 0.5⩽ as a borderline; synergy was detected with tigecycline-colistin methanesulfonate combination. It should be pointed out that additive interaction was more frequent. No antagonism was observed.
Conclusion: Tigecycline exhibited potent in vitro antibacterial activity against clinical *A. baumannii* strains. The results of combination studies for the carbapenem resistant *A. baumannii* provide evidence that tigecycline used in combination with colistin methanesulfonate will produce synergy against studied strains.

R2133 In vitro activity of tigecycline against multidrug-resistant Enterobacteriaceae clinical isolates

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Objective: To evaluate the in vitro activity of tigecycline against 108 multidrug-resistant (MDR) strains of Enterobacteriaceae isolated from ICU and non-ICU patients of our hospital.
Methods: A total of 108 MDR strains of Enterobacteriaceae collected over a 10-month period, were studied. The minimum inhibitory concentrations (MICs) for tigecycline and 14 other antimicrobial agents were determined by the E-test method (AB Biodisk, Sweden). EUCAST breakpoints were used to interpret tigecycline and colistin MIC results and CLSI (M100-S18) breakpoints were used to interpret all other agents, where applicable.
Results: Bacterial species identified were: *K. pneumoniae* (77/108), *E. coli* (20/108), *Proteus mirabilis* (7/108), *E. cloacae* (3/108) and *K. oxytoca* (1/108). They were isolated from urine (38/108), blood (21/108), bronchoalveolar lavage fluid (BAL) (19/108), pus (8/108), intravenous catheters (6/108), and others (16/108). MIC50 and MIC90 for tigecycline was 1 and 2 mg/l, respectively (MIC range: 0.125–4 mg/l). All *E. coli* isolates tested (MDR, ESBL and MBL producing) were uniformly sensitive to tigecycline. Resistance to tigecycline was detected

in 1 of the MDR *K. pneumoniae* isolate (1.3%), and in all 7 *P. mirabilis* isolates tested.
Conclusion: The present data suggest that tigecycline may be an effective option for treatment of infections caused by MDR Enterobacteriaceae (with the exception of *Proteus mirabilis*) in our setting.

R2134 The challenge of multiresistant Gram-positive cocci: novel, effective therapeutic options

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Introduction: Multiresistant Gram-positive cocci, including *Staphylococcus aureus*, the group of coagulase-negative staphylococci, *Enterococcus faecalis* and *Enterococcus faecium*, as well as *Streptococcus pneumoniae* and other streptococci, represent emerging pathogens in the community, but especially in the setting of immunocompromised, hospitalised patients.
Methods-Results: In these last conditions of elevated risk, multiresistant Gram-positive infections occur in particular when surgery, invasive procedures, or prosthetic implants are of concern, patients are admitted in intensive care units, or underlying chronic disorders and immunodeficiency are of concern, and broad-spectrum antibiotics are widely used in the environment. The spectrum of antimicrobial compounds now available for an effective management and treatment of these relevant infections is significantly threatened by the emerging and spread of methicillin-resistant and more recently glycopeptide-resistant microbial strains. The streptogramine association represented by quinupristin/dalfopristin, the oxazolidinone derivative linezolid, and the recently licensed lipopetide daptomycin and the glycylcycline tigecycline, together with a number of novel glycopeptides (including the once-weekly dalbavancin), novel fluoroquinolones, novel cephalosporins with a spectrum including also methicillin-resistant staphylococci, and a number of experimental compounds on the pipeline, represent an effective response to the majority of these problems, due to their innovative mechanisms of action, their maintained or enhanced activity against multiresistant pathogens, their effective pharmacokinetic/pharmacodynamic properties, their frequent possibility of synergistic activity with other compounds effective against Gram-positive pathogens, and a diffuse potential for a safe and easy administration, also in the setting of compromised patients.
Conclusion: The most relevant microbiological, pharmacological, and therapeutic issues related to the epidemiology of multiresistant Gram-positive infection, the potential clinical indications of all recently available compounds compared with the standard of care of treatment of resistant Gram-positive infections, and an updated overwiew of data on efficacy and tolerability of all these compounds and those on advanced investigation, are updated and outlined on the ground of an extensive review of all available, recent evidences coming from clinical trials figures and the international literature.

R2135 A mini-cluster of chronic bone-joint-prosthetic-soft tissue infection due to multiresistant *Acinetobacter baumannii* strains in orthopaedics setting. Effective treatment with tigecycline plus colistin

R. Manfredi (Bologna, IT)*

Introduction: *Acinetobacter* spp is an environmental organism characterised by a proportionally low intrinsic virulence, but a concurrent, broad-spectrum and high-level resistance to the majority of available antimicrobials.
Methods and Results: Six unrelated cases of *A. baumannii* infection interesting bone, joint, prosthetic devices, and close soft tissue occurred during Oct 2007-Jun 2008, at a specialised Orthopedics Hospital of Bologna, Italy. In all cases a concurrent, local polymicrobial infection including Gram-positive organisms (Staphylococci-Enterococci), and Gram-negative agents (*Escherichia coli*, *Citrobacter freundii*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*), was diagnosed during the long-term admission (32–112 days). While concurrent microorganisms were controlled by appropriate chemotherapy and the implant of antibiotic-impregnated cement, *A. baumannii* showed complete resistance to an enlarged panel of antiinfective compounds, save colistin and tigecyclin (the only agents with MIC values <0.5 μg/mL). As a consequence, both these compounds were administered at full dosage and by i.v. route, in association with carbapenems, rifampicin, amikacin, and cotrimoxazole, to exploit the potential synergistic activity against *A. baumannii* demonstrated by in vitro studies. In the meantime, an active, systematic surveillance program interested the entire Hospital (with special focus on surgery rooms, intensive care units, and diagnostic-therapeutic devices), but no apparent foci of environmental colonisation were found. The micro-cluster of 6 cases of multiresistant *A. baumannii* infection was cured after 16–61 days of combined colistin-tigecycline therapy, and neither relapses nor novel episodes occurred in subsequent 4 months.
Conclusions: Both the oldest and the newest compounds play a crucial role in the treatment of multiresistant infections caused by challenging pathogens like *A. baumannii*. A 60-year-old drug like colistin, with a new molecule like tigecycline, perfectly traced the target organism, and successfully resolved long-lasting infections burneded by multiple morbidity, forced patients' inability, and extremely prolonged admission times. The selection of eventual, synergistic agents may be conducted empirically, or better with the aid of enlarged in vitro susceptibility assays. A strict co-operation among specialistic surgeons, Infectious Diseases consultants, Microbiologists, and Pharmacists, is warranted to contain the impact of these increasing environmental infections.

R2136 Antimicrobial activities of bacteriocins E50–52 and B602 against MRSA and other nosocomial infections

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Our objective was to determine the antimicrobial activities of previously published bacteriocins E50–52 and B602 against methicillin resistant *Staphylococcus aureus* (MRSA) and other prominent nosocomial bacterial infections.
Methods: Several Russian hospitals were enlisted into the study from 2003 to 2007 and the hospitals supplied isolates of *Acinetobacter* spp. (n = 11), *Citrobacter freundii* (n = 8), *Escherichia coli* (n = 9), *Klebsiella pneumoniae* (n = 10), *Proteus* spp. (n = 6), *Pseudomonas aeruginosa* (n = 10), and *Staphylococcus aureus* (n = 10). Susceptibilities were determined by Kirby-Bauer disc-diffusion assays using 12 antibiotics belonging to different functional groups. Minimal inhibitory concentrations (MICs) were determined for 19 antibacterials. Extended spectrum β-lactamase (ESBL) genes were detected using a standardised polymerase chain reaction (PCR) method.
Results: The amino acid sequences, molecular weights and the isoelectric points of both bacteriocins E50–52 and B602 were determined as being consistent with class IIa characteristics, containing 39 and 29 amino acid residues, molecular weights of 3,932 and 3,864 Da, and pI values of 8.5 and 7.2, respectively. ESBL genes were detected in 27 of the 32 *C. freundii*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis* isolates and, were not detected in the *Acinetobacter* spp., *Ps. aeruginosa* or *S. aureus* isolates. The antibiotic profiles of the 64 diverse isolates manifested substantial resistance, as associated with the poor clinical outcomes of the infected individuals. The MIC values of the same isolates ranged from <0.025 to 1.56 μg/ml for bacteriocin B602 and 0.05 to 6.25 μg/ml for bacteriocin E50–52.
In conclusion, for the 64 diverse clinical isolates tested, antibiotic resistance was unfortunately high while the susceptibility to the bacteriocins tested was remarkably good. The potentials for application of bacteriocins in clinical settings as therapeutic agents appear quite promising.

R2137 In vitro activities of tigecycline and other 22 antimicrobial agents against *Streptococcus pneumoniae* strains isolated in a tertiary care hospital

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Objective: Tigecycline is a glycylcycline with promising broad-spectrum activity, including resistant Gram-positive organisms. We compared the in vitro activities of tigecycline to those of other antimicrobial agents against 105 non-duplicate isolates of *Streptococcus pneumoniae* recovered from patients treated in the University Hospital of Heraklion, Crete, Greece in a 27-month period.
Methods: Pneumococci were identified using standard techniques, including Gram stain characteristics, colonial morphology, optochin susceptibility and bile solubility.
MICs of penicillin, cefuroxime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, erythromycin, clarithromycin, azithromycin, roxithromycin, ciprofloxacin, levofloxacin, moxifloxacin, sparfloxacin, tetracycline, chloramphenicol, cotrimoxazole, vancomycin, linezolid, quinupristin-dalfopristin, vancomycin and tigecycline were determined by using the E-test method (AB Biodisk, Solna, Sweden). *Streptococcus pneumoniae* ATCC 6305 and ATCC 49619 were used as control strains.
Results: Among these isolates, 41.9% were penicillin nonsusceptible, with 21.9% being penicillin-intermediate (PISP) and 20% being penicillin-resistant (PRSP). Resistant rates (intermediate and resistant) among non-β-lactam agents were as follows: macrolides, 30.5%; clindamycin, 10.5%; tetracycline, 29.5%; chloramphenicol, 0.9%; and trimethoprim-sulfamethoxazole, 23.8%. Resistance to multiple antimicrobial agents was observed among 27 (61.4%) PRSP and PISP strains. No resistance to vancomycin, linezolid, quinupristine/dalfopristin and tigecycline was observed. MIC50 and MIC90 values of tigecycline were found to be 0.032 and 0.047 mg/l respectively, with a range of 0.016–0.125 mg/l.
Conclusion: Tigecycline demonstrated excellent in vitro activity against both penicillin-sensitive and penicillin-resistant strains. It might thus represent an alternative treatment for infections caused by multiresistant *Streptococcus pneumoniae*.

R2138 Novel antifungal phenolic compound from *Parrotia persica*

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Aqueous and methanol extracts of *Parrotia persica* leaves were assayed for antifungal activity against phytopathogenic *Fusarium oxysporum* and human pathogenic *Candida albicans* by poisoned food technique. Both the aqueous and methanol extracts demonstrated significant antifungal activity. Further fractionation of methanol extract guided by antifungal activity resulted in the isolation of an active principle which was identified as phenolic compound by phytochemical analysis. The structure of the active principle was elucidated by Mass spectroscopy, 1H NMR and 13C NMR spectroscopy. These results revealed that the compound is 6-(ethoxymethyl)-tetrahydro-2H-pyran-2, 3, 4, 5-tetraol compound with 1-isopropyl-4-methoxybenzene. The compound was found responsible for antifungal activity against both *F. oxysporum* and *Candida albicans*.

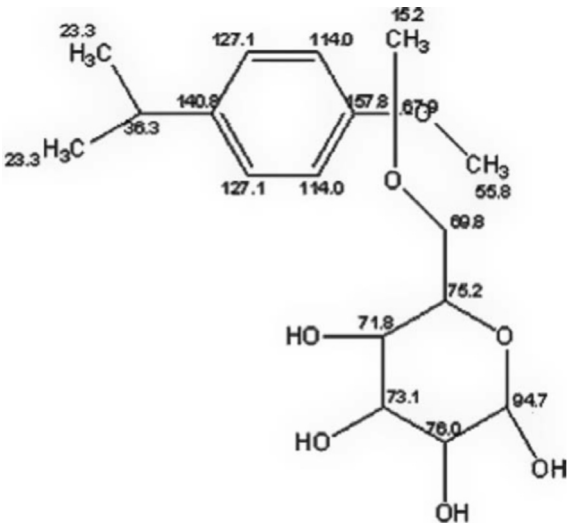

Figure: Structure of the active principle [6-ethoxymethyl)-tetrahydro-2H-pyran-2,3,4,5-.

Epidemiology of MRSA, VRE and other Gram-positives

R2139 Prevalence, antimicrobial resistance and clonal features of drug-resistant *Enterococcus faecalis* and *E. faecium* colonising high-risk patients in a Portuguese hospital

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Objectives: To establish the prevalence, antimicrobial resistance and clonal features of *Enterococcus faecalis* – Efl – and *E. faecium* – Efm – resistant to gentamicin-120 (CN120) and glycopeptides (GR) colonising high-risk patients in a Portuguese hospital during a four months period (2006–07).
Methods: The VITEK2 system was used for microbial identification and antimicrobial susceptibility testing. The vanA/B and aac(6′)-aph(2″) genes were detected by PCR in all GR and CN120 Efl and Efm. The asaI, cylA, gelE, hyl and esp virulence genes were screened by Multiplex-PCR. SmaI-PFGE was used for clonal assessment. GR isolates were tested by multilocus sequence typing (MLST).
Results: A total of 108 haematological malignancy patients were screened weekly, generating 275 rectal swabs and 212 enterococci mainly Efl (53.8%), and Efm (35.8%). Close to 50% (39/76) of Efm and 32% (36/114) of the Efl were multiresistant: (i) Efm resistant to CN120, ampicillin-Amp, erythromycin-E, clindamycin-Da, ciprofloxacin-Cip, 6 of 39 were also resistant to GR with vanA genotype. (ii) Efl resistant to CN120-E-Da-tetracycline-quinupristine/dalfopristin and 21 of 36 were resistant to Cip. Two out of 7 Efl PFGE patterns were dominant: PFGE AO (19/36 isolates) and PFGE A (11/36 isolates). Most of the Efl PFGE AO (84%, 17/19) carry asaI-cylA-gelE-esp genes, and 88% were gelatinase producers, while 73% (8/11) of the isolates belonging to PFGE A had the asaI-cylA-esp profile. The majority of the Efm isolates (92%, 36/39) belonged to the dominant PFGE c. The esp gene alone or with hyl gene were detected among 58% (21/36) and 25% (9/36) respectively of the Efm isolates included PFGE c. All but one Efm-GR belong to the genetic lineage ST17. ST280 was associated to a single Efm isolate of PFGE x, that lacks the virulence genes tested. The two Efl and Efm dominant clones (PFGE AO and PFGE c, respectively) colonising the patients were also detected among enterococci resistant to CN-120 and/or GR recovered from clinical samples of this same hospital where became epidemic during 2004–2006.
Conclusions: These data provide further insights on the dynamics of colonisation by multidrug-resistant enterococci in a particular population of haematological malignancy patients, who may be used to monitor enterococcal carriage of epidemic and persistent clones useful for infection control measures.

R2140 Prevalence of methicillin-resistant *Staphylococcus aureus* among students of the Faculty of Veterinary Medicine in Bari, Italy

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Objectives: Recent epidemiological studies have demonstrated that veterinary students and veterinarians may have higher risks of exposure to Methicillin resistant *Staphylococcus aureus* (MRSA) than the general population. The aim of the study was to analyze the prevalence of MRSA among students of a Faculty of Veterinary Medicine (Bari, Italy).

Methods: From November 2007 to January 2008, 200 students of the Faculty of Veterinary Medicine were randomly selected from the 2nd, 3rd, 4th and 5th year. The students were asked to provide a nasal swab and to fill a questionnaire inherent the contact with animals (vet-care associated factors) and exposure to well-known MRSA risk factors (health-care associated factors). The samples were subjected to bacteriological analysis and PCR for the mec A gene. The data collected with the questionnaire were evaluated by statistical analysis (chi square test for the dichotomous variables and univariate analysis for the risk factors).
Results: The response rate to the study was 84% (168 out of 200 samples). Twenty-two out of 168 students (13.1%) were found to be MRSA carriers. Nine out of 22 MRSA carriers had either relatives working in health-care facilities or subjected recently to hospitalisation. Fifteen out of 22 carriers attended the 4th and 5th years of the course. Statistical analysis of the risk factors showed that the ratio of MRSA carriers increased with having one or more healthcare-workers in the family (Odds ratio, OR 5.8, 95% Confidence Interval, CI 1.7–20.4) and with being admitted to the 4th and 5th years of the course (OR 3.9, 95% CI 1.5–10.1), when the students are expected to make practice with animals.
Conclusion: The high prevalence of MRSA carriers among students of the Veterinary Faculty of Bari may be linked both to health-care and vet-care associated factors (chiefly making practices with animals). Molecular characterisation of the MRSA isolates will help elucidate more accurately the origin and relationships of the strains.

R2141 Spread of epidemic vancomycin-resistant *Enterococcus faecium* clonal complex 17 in haematological patients in Russia

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Objectives: In this study we investigate molecular background of selected epidemic vancomycin-resistant (VR) *E. faecium* isolated from haematological patients in Russia by Multilocus Sequence Typing (MLST).
Methods: Genetic relatedness of VR *E. faecium* collected during 2004–2007 was examined by Pulsed-field Gel Electrophoresis (PFGE) using SmaI. Predominant epidemic clones A (A1-A30) and F (F1-F8) were determined. Further MLST analysis was performed on a subset of 16 isolates representing subclones that have spread most widely (A1, A3, A8, A10, A16, A26, F1, F3). Detection of virulence genes esp, gelE, cylA, hyl, and agg was performed by PCR.
Results: MLST analysis revealed that all isolates belonged to the clonal complex 17 (CC17) of hospital adapted, epidemic strains. Strains grouped into three sequence types (STs): ST202 (n = 11), ST18 (n = 3) and its single locus variant ST262 (n = 2). ST202 was first detected in 2004. It persisted during 2005–2006 together with ST18. In 2006, ST262 appeared probably arising from ST18. Relatedness of ST18 and ST262 is confirmed by the same virulence patterns: strains harboured two genes esp and hyl simultaneously. All of ST202 isolates also had a fixed virulence profile. These strains possessed epidemicity marker esp and a potential virulence gene gelE, which is rarely found in *E. faecium*.
Conclusion: MLST of VR *E. faecium* from haematological patients in Russia revealed the spread of CC17, associated with nosocomial infections worldwide. Our results demonstrate presence of virulence gene esp, a marker for epidemicity in different clonal types and its persistence in time.

R2142 Endocarditis due to vancomycin-resistant enterococci (*E. gallinarum*): a case report

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The first described vancomycin resistant enterecocci (VRE) was about twenty years ago. Recently many clinics reported VRE. However endocarditis due to VRE is still a rare entity and there are only a few cases reported in the literature.
Case: We report a 59 year-old male patient with chronic renal failure who was on haemodialysis. He presented with a sudden onset of fever, tachycardia and respiratory distress. Echocardiography was performed and vegetation on the mitral valve was found. As he was diagnosed to have endocarditis the patient was put on ampicilin and gentamicin therapy. He underwent an emergent mitral and aortic valve surgery due to heart failure. While he was still on ampicilin and gentamicin therapy, *E. gallinarum* that was resistant to vancomycin (MIC > 32 mg/L) was isolated from the surgical valve specimens and hence his antibiotic regime was switched to teicoplanin (MIC=0.5 mg/L). 28 days after teicoplanin therapy the patient was discharged with free of symptoms and any complication.
Conclusion: Endocarditis due to vancomycin resistant enterococci is an uncommon clinical situation. In review of the literature the commonly encountered causative agent for endocarditis was found to be *E. faecium* especially in patients with comorbid conditions. This patient had no prior known valvular disease therefore having no cardiac predisposition but presented with endocarditis caused by *E. gallinarum*. This patient is presented as an example for a sudden onset endocarditis with an unusual type of enterococci.

R2143 Increased rate of methicillin resistance among *Staphylococcus aureus* isolates at a major general hospital located in northern Italy

R. Manfredi, A. Nanetti (Bologna, IT)*

Introduction: The increased rate of methicillin resistance among Gram-positive cocci is a general concern, especially in the hospital setting. A prospective microbiological monitoring including a continued surveillance of antimicrobial susceptibility rates, is ongoing at our General Hospital, located in Bologna, Italy.
Materials and Methods: The temporal variations of in vitro antimicrobial sensitivity figures were examined quarterly for all suitable *Staphylococcus aureus*, during the year 2007. The same pathogen cultured more than once from the same patient within one month, has been considered one time only.
Results: Among *Staphylococcus aureus* isolates (357 strains tested on the whole), both available glycopeptides (vancomycin and teicoplanin), maintained a full 100% sensitivity profile over time, while the rate of methicillin resistance showed a significant growth from January-March 2007 (40.7% of tested strains), to April-June 2007 (47.5%), to July-September (42.2%), up to October-December 2007 (56.0% of tested strains) ($p < 0.01$). When considering antibiotics other than β-lactam ones, cotrimoxazole maintained a consistently elevated activity over time (92.3% to 100% of microbial strains tested during the study period), followed by choramphenicol (82.7% to 87.8% of tested strains), and rifampin (67.6% to 73.5% of tested strains), while clindamycin showed a worse sensitivity profile (48.1% to 57.9% of tested strains), as well as erythromycin (48.8% to 57.9% of tested strains), and gentamicin (40.4% to 52.5% of tested strains). Among antimicrobial compounds other than β-lactam derivatives, no significant rates of antibiotic susceptibility rates were observed during the study period, against *Staphylococcus aureus* isolates.
Conclusions: A prospective bacteriological surveillance of antimicrobial susceptibility rates of a major hospital-associated microorganism like *Staphylococcus aureus* is relevant, to establish reliable guidelines of antibiotic treatment and prophylaxis, on both local and regional basis. Despite a significant increase of methicillin resistance rates, "older" compounds like cotrimoxazole, chloramphenicol, and rifampin, may still play a role in selected clinical instances, while the activity of vancomycin and teicoplanin remains fully preserved until now.

R2144 Microbiological features of *Enterococcus faecalis* and *Enterococcus faecium* assessed according to a hospital-based prospective surveillance programme: in vitro antimicrobial susceptibility profile, and temporal trend

R. Manfredi, A. Nanetti (Bologna, IT)*

Introduction: The increased temporal rate of antimicrobial resistance among Gram-positive cocci (including Enterococci) is a major concern, especially in hospital-based settings.
Materials and Methods: The temporal trend of the in vitro antibiotic susceptibility rates was investigated for all *Enterococcus faecalis* and *Enterococcus faecium* strains, isolated at our tertiary-care Hospital during the year 2007. The same pathogen isolated more than once from the same patient within one month, has been considered once.
Results: Among *Enterococcus faecalis* isolates (705 strains tested on the whole), the greater activity rate was achieved by linezolid (100% of tested strains), followed by nitrofurantoin (97.4–100% of strains), teicoplanin (94.8–100%), vancomycin (87.5–100%), ampicillin (89–92.4%), penicillin (87.9–91.0%), while appreciable, but irregular variations of sensitivity occurred over time for gentamicin, streptomycin, and tetracyclines. With regard to *Enterococcus faecium* strains (175 strains), both linezolid and teicoplanin maintained a 100% in vitro activity, followed by vancomycin (86.4–100% of strains), streptomycin (62.5–100%), gentamicin (52.9–63.6%), and tetracyclines (51.8–49.6%), while negligible efficacy was shown by ampicillin (7.5–18.5% of tested strains) and penicillin (7.5–18.5%). Eighteen strains of vancomycin-resistant *Enterococcus faecalis* strains were detected (12 concentrated in the July-September period), while vancomycin-resistant *Enterococcus faecium* strains were six through the entire observation year. No significant temporal modifications of antimicrobial sensitivity rates were observed, as well as no significant change in the emergence of vancomycin-resistant strains.
Conclusions: A prospective surveillance monitoring of in vitro antimicrobial susceptibility rates of some relevant hospital-associated organisms like Enterococci represents an useful tool to address antibiotic treatment and prophylaxis, on local and regional basis. The emerging of resistance to the reference compounds like glycopeptides may be also well targeted on these basis, in order to preserve the clinical use of the majority of molecules which still guarantee effective activity of these difficult-to-treat Gram-positive cocci.

Antibiotic usage

R2145 Impact of an antimicrobial stewardship programme on antimicrobial consumption and cost in an Italian hospital

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Objective: To evaluate the impact of an Antimicrobial Stewardship Program (ASP) on consumption and cost of antimicrobials.
Methods: from January 2008, an ASP has been carried out in our 1400-bed referral hospital in the North-Eastern Italy by ID consultants, pharmacists and microbiologists, who constitute the Antimicrobial Management Team (AMT). Antimicrobial formulary restrictions (mainly concerning broad spectrum- and last generation-antibiotics), microbiologically-driven empirical and targeted therapies, and regular educational meetings were the most important interventions implemented. We initially analyzed two surgical and three medical wards (vascular/thoracic surgery, urology, 2 internal medicine divisions and pneumology). Defined daily doses (DDD)/100 bed-days consumption pooled data were analyzed through the ESGAP ABC Calc 3.1b program for the year 2008. These data were then compared to the same data of 2007, in which the ASP had not yet been implemented.
Results: a remarkable decrease of restricted antimicrobials consumption has been observed between the two years, whereas a comparable trend was not detected for freely available drugs. Moreover, drug acquisition costs strongly decreased. The main results are summarised in the table, where "*" indicates some of the restricted antimicrobials at our institution.
Conclusions: although preliminary and concerning a limited number of wards of our hospital, these data strongly support the need for a multidisciplinary approach to antimicrobial therapy at institutional level to decrease antimicrobial pressure and to control expenditures. Through the regular activity of the AMT, this integrated approach may also affect the appropriateness of antimicrobial prescription within the whole hospital.

Antibacterial	DDD		EUR		
	2007	2008	2007	2008	2007/2008
Cefuroxime	3,092.3	3,125.0	17,228.73	17,048.64	−180.09
Cefotaxime	3,026.0	2,482.0	14,777.69	7,374.42	−7,403.27
Ceftazidime*	354.8	163.8	6,319.77	2,819.37	−3,500.40
Cefepime*	105.0	8.0	919.25	69.99	−849.26
Meropenem*	410.0	126.8	17,343.49	5,422.43	−11,921.06
Imipenem/enzyme inhibitor	375.8	202.8	16,307.81	8,652.44	−7,655.37
Ciprofloxacin	1,655.2	1,702.8	55,725.92	44,533.39	−11,192.53
Levofloxacin	2,758.0	3,479.0	72,583.33	91,820.62	+19,237.29
Vancomycin*	341.3	199.3	2,592.07	1,402.68	−1,189.39
Teicoplanin*	501.0	195.5	18,070.01	7,079.17	−10,990.84
Total			221,868.07	186,223.15	−35,644.92

R2146 Effectiveness of an antimicrobial stewardship program on broad-spectrum antimicrobials in a Hong Kong tertiary-care hospital

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Objectives: A multidisciplinary Antimicrobial Stewardship Program (ASP) with an interventional management team (AMT) was implemented in a 1350-bed teaching hospital in Hong Kong in 2006. Its impact on the appropriateness of prescription, consumption, resistance and clinical outcomes in patients of the medical department was examined.
Methods: 635 patients, prescribed one of eight targeted agents, vancomycin, teicoplanin, carbapenems, linezolid, cefepime, ceftazidime, cefoperazone/sulbactam and piperacillin/tazobactam during February to August 2006, were reviewed prospectively. 605 cases prescribed these agents during March-May 2005 at pre-intervention were reviewed.
Results: Antibiotic prescription of these targeted agents was reduced from 83 to 63 prescriptions/1000 admissions after ASP implementation. Inappropriate use/recommendations were made in 19% of cases. An increase in appropriate prescriptions was obtained during the intervention period (O.R. 3.15; 95% C.I. 2.07–4.79, $p \leqslant 0.001$). The length of hospital stay in the two groups was not different but a reduction of 3.6% in all cause mortality was observed in the intervention group. The consumption of targeted antimicrobials in defined daily doses (DDD)/1000 bed days occupied (BDO) was reduced by 4.5% overall in 2006. There was a significant reduction in the resistance rates of *Escherichia coli* to amoxicillin/clavulanate, 35.28 vs 30.84; $p = 0.02$ for all specimens, 47.8 vs 29.56; $p = 0.006$ for blood culture specimens, but no significant change in rates of MRSA or ESBL Gram negative bacteria was observed.
Conclusion: The implementation of ASP in the hospital resulted in more rational use of antimicrobials, an overall reduced consumption of antibiotics and improved clinical outcomes.

R2147 Optimisation of systemic antimicrobials usage in multi-profile hospitals in Russian Federation

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Objectives: Insufficient data on systemic antimicrobials (AM) consumption in Russian Federation (RF) appears to be the main barrier for assessing and optimising AM use in inpatient settings. Hence we aimed our study to develop an on-line program of systemic AM use monitoring for hospital wards with intensive AM usage in different regions of RF.
Methods: On-line database development used products and technologies consisted of operation system (Microsoft Windows Server 2003),

web framework (ASP.NET 2.0), database (Microsoft SQL Server 2005), development IDE (Microsoft Visual Studio 2008). ATC/DDD methodology was used as a basis for data presentation and calculation of AM consumption.
Results: The database consisted of two parts. The first part contained information about AM package numbers and costs based on charges invoices from hospital pharmacy could be collected on a quarterly basis at the department level. The AM consumption is expressed as number of DDD per 100 bed-days. Distribution of AM use according to patients' profile (first, second choice, etc.) on physician judgment could also be performed. This part of the program allows getting an instantaneous report on AM consumption and ABC-analysis of AM expenditure immediately after data entry. The second part of the database is designed to collect information on any AM prescription with data binding to the patient's characteristics. The data about demographic characteristics of patients (gender, age), diagnosis (main diagnosis, complications, concomitant diseases), AM trade and generic names, dosing regimen, route of administration, duration of treatment, reason for AM use (prophylaxis or therapy with specification of diagnosis/procedure) and AM discontinuation/modification, any adverse reactions, length of hospital stay and treatment outcome could be gathered and analyzed.
Conclusion: On-line registration will facilitate data processing and afford an opportunity to make a decision on AM administration practice and/or AM purchases modification in time. Detailed analysis of AM use will allow establishing a correspondence between practice and existent standards and developing specific programs aimed at AM use optimisation and reduction of unreasonable expenses.

Molecular bacteriology

R2148 A molecular diagnostic approach for MRSA hospital outbreaks providing all necessary data of patients and contacts within the first 24 hours

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During MRSA hospital outbreaks a rapid and accurate detection of patients infected or colonised with the outbreaking strain is crucial. However, the standard culture method for detecting MRSA is time-consuming, taking several days. Therefore, recently, several MRSA PCR detection methods are being developed to accelerate the MRSA tracing. We designed a procedure combining two MRSA PCR detection methods, to manage all MRSA outbreaks within 24 hours, using the in house MecA/Martineau PCR developed by Van Hannen, Anthonious Ziekenhuis, Nieuwegein and the commercially available IDI MRSA PCR (based on the Huletsky primers).
After an overnight culture incubation step the MecA/Martineau PCR is used as a first step. Positive results are confirmed in the more specific Huletsky PCR.
Since the introduction of our "24 hours all-in" procedure we were able to manage several MRSA outbreaks in three different hospitals. A total of 396 out of 4086 clinical samples (9.7%) appeared to be positive in the MecA/Martineau screening PCR and thus needed further processing with the IDI MRSA PCR.
Of these samples 28.8% (114/396) were also positive in this Huletsky PCR. In these remaining 114 samples MRSA could be cultured in 89.
In conclusion, this approach combines the advantage of a high throughput, which is essential in outbreak situations, with a superior sensitivity and specificity through the combination of PCR's targeting different parts of the genome and is therefore able to give an exact overview of the spread of MRSA within 24 hours after receiving the samples.

R2149 Study of vac A genotypes of *H. pylori* isolated from patients with upper gastrointestinal diseases by PCR

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Background and Objectives: *Helicobacter pylori* is a Gram-negative curved bacilli which is colonised in the human stomach. It causes duodental ulcer, gastritis and is associated with adenocarinoma. There might be a possible relationship between cagA, vacA and ice a genes and clinical outcomes. The aim of this study was to identify the frequency od vacA genotypes of *H. pylori* isolated from the upper gastrointestinal.
Materials and Methods: 100 *H. pylori* strains were isolated from patients with different gastrointestinal disease in Azahra hospital. Vac A alleles were typed using PCR with specific primers.
Results: There were four Vac A mosaicsms, including 28 for s1a/m1 (28%), 23 for s1b/m1 (23%), 26 for s1a/m2 (26%) and 23 for s1b/m2 (23%), s2 form was not found.
Conclusion: The results showed there is no significant relationship between different genotypes of vacA and the related diseases.

R2150 Comparison of 16S rRNA, 23S rRNA and gyrB genes sequences in phylogenetic relationships of *Shigella* isolates from Iran

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Objectives: *Shigella* is one of the most important agents of acute diarrhoea and gastrointestinal disorders worldwide that cause bacillary dysentery which remains a significant threat to public health with high rate of death. Since differentiation of *Shigella* isolates from each other is difficult, some reports indicate that application of 16S/23S rRNA and gyrB genes sequences is a useful method for this purpose. Based on our knowledge it is the first report to differentiate *Shigella* species in Iran by these genes. The homology rate of Iranian *Shigella* strains was compared with each other in this study.
Methods: Twenty *Shigella* isolates from diarrhoeal patients were studied. They were cultured on XLD and MacConkey agar. Biochemical and serotyping tests were done to distinguish *Shigella* strains. DNA was extracted by phenol-chloroform method. Complete gene of 16S rRNA (1542 bp), 23S rRNA (2904 bp) and partial gene of gyrB (1256 bp) were amplified by PCR, and then were sequenced by ABI3130X1. We used CLUSTAL W software to align nucleotide sequences. A neighbour-joining analysis was performed to reconstruct phylogenetic tree with the MEGA 3.1 software.
Results: The homology rate based on both 16S rRNA and gyrB gene sequences between each 5 isolates of *Shigella sonnei* and *Shigella boydii* were 100% and 99.9% respectively. Based upon gyrB gene sequence, each 5 isolates of *Shigella flexneri* and *Shigella dysenteriae* showed 99.6% and 99.5% similarity, whereas they showed 99.8% and 99.9% similarity by 16S rRNA gene sequence. The phylogenetic tree based on 23S rRNA indicates that the homology between each specimens of *S. flexneri*, *S. boydii*, *S. dysenteriae* and *S. sonnei* were respectively 99.4%, 99.7% 99.3% and 100%.
Conclusion: Phylogenetic-tree analysis is often used as a method to classify organisms. Our data indicates that, although the 16S rRNA sequence method is a highly accurate and rapid method for identifying most bacteria to the genus level, the gyrB sequence method might be more useful for identifying bacteria to the species level. In particular, 23S rRNA analysis of bacteria is an effective means to classify closely related species. Our data were in accordance with the previous studies reported from other countries, indicating close relationship among *Shigella* species isolated from Iran.

R2151 Genetic heterogeneity of emm12 genotype *Streptococcus pyogenes* strains isolated in St. Petersburg, Russia

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Objectives: *Streptococcus pyogenes* or group A streptococci (GAS) is a leading cause of human infections varied from mild pharyngotonsillitis to severe invasive diseases. GAS being the polylysogenic bacterium can contain from 2 to 6 different temperate bacteriophages that provides genetic heterogeneity to this specie. It is supposed that the expression of the certain toxins encoded by prophage genes correlates with virulent properties of GAS strains. The aim of the present study was to characterise the presence of phage-associated genes, including genes for the toxins and integrases, in GAS strains of emm12 genotype isolated in Saint-Petersburg.
Methods: The collection of 172 GAS strains isolated in Saint-Petersburg (Russia) in 2006–2007 was used. The emm genotyping of 64 randomly selected epidemiologically unrelated strains was done according to recommendation of the Centre for Disease Control. Chromosomal DNA of the strains was isolated by the phenol/chloroform extraction. The presence of 19 phage-associated genes (Table 1) was analyzed by PCR.

Table 1. Distribution of the phage gene profiles among the 18 GAS strains of *emm12* genotype

Phage gene	Encoded protein	*emm* subtype							
		emm12.0, no. of strains							*emm12.22*
		10	2	1	1	1	1	1	1 strain
speA	Toxin A	–	–	–	–	+	–	+	–
speI	Toxin I	+	+	+	–	+	+	+	+
speH	Toxin H	+	+	+	–	+	+	+	+
speL	Toxin L	–	–	–	–	–	–	–	–
sda	Streptodornase	–	–	–	–	–	–	–	–
speCJ	Toxin C variant J	–	–	–	+	+	–	–	–
ssa	Superantigen	–	+	–	–	–	+	+	–
speC	Toxin C	+	+	+	–	+	+	+	–
sla	Phospholipase A2	–	–	–	–	–	–	+	–
smeZ	Toxin Z	+	+	+	+	+	+	+	+
int1	Integrase 1	–	–	–	–	–	–	+	–
int2	Integrase 2	–	–	–	–	–	–	+	–
int3	Integrase 3	+	+	–	–	+	–	+	–
int4	Integrase 4	–	+	–	–	+	+	+	–
int5	Integrase 5	+	+	+	–	+	+	+	+
int6	Integrase 6	–	–	–	–	–	–	–	–
int7	Integrase 7	–	–	+	+	+	–	+	–
int8	Integrase 8	–	–	–	–	–	–	+	–
int9	Integrase 9	–	–	–	–	–	–	–	–

Symbols "+" and "–" indicate the presence or absence of the corresponding phage gene in the genome of GAS strain.

Results: Among the 64 GAS strains under study, 18 strains belonged to emm12 genotype. They were characterised by the emm12.0 (17 strains) and emm12.22 (1 strain) subtypes. The toxin genes smeZ, speI, speH, speC were predominant among the strains. They were revealed in 18, 17, 17 and 16 strains, respectively. The genes speL and sda were not found, and the genes sla and speC/J were present in 1 and 2 strains, respectively (Table 1). The integrase genes int5 and int3 were found in 17 and 14 strains, respectively, while the genes int6 and int9 were absent in the strains under study. Presence of the toxin speI and speH genes correlated with the presence of int5 gene. Most of the strains contain 2 or 3 integrase genes, that indicates the presence of 2 or 3 temperate bacteriophages in their genomes (Table 1). At the same time a single strain contained seven integrase genes, and 2 strains contained one integrase gene each. A total of 8 different profiles of the phage-associated genes were revealed among the 18 GAS strains of emm12 genotype, including 7 profiles among the 17 strains of emm12.0 subtype (Table 1).
Conclusion: The strains of emm12 genotype isolated in Saint-Petersburg in 2006–2007 are characterised by significant genetic heterogeneity. Analysis of the prophage gene content is valuable approach for molecular epidemiology of *Streptococcus pyogenes* infections.

R2152 Use of the GenXpert system for detection of *S. aureus* and MRSA directly in clinical samples

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Objectives: Early microbiological documentation is a key point for adapted antibiotherapy in patients with a septic shock. This is difficult to achieve using conventional bacteriological methods, requiring at least 36–48 h to obtain final results. Real-time PCR could provide an earlier identification of the pathogen with a better sensitivity. Methicillin-sensible and methicillin-resistant *Staphylococcus aureus* (MSSA and MRSA) are frequently involved in severe sepsis of nosocomial origin community-acquired.
The Xpert® MRSA/SA Blood Culture kit (Cepheid, Instrumentation Laboratory) used in conjunction with the GeneXpert real-time PCR platform (Cepheid, Instrumentation Laboratory) has been developped to allow the rapid detection in 50 min of *S. aureus* and MRSA in blood cultures. This assay does not require sample preparation time. The present study evaluated this kit for use directly from clinical samples.
Method: We tested 27 clinical samples (13 respiratory samples, 4 discharge specimen, 1 eschar swab, 5 urines, 3 catheters, 1 serum) from which *S. aureus* strains were isolated, 6 MRSA and 19 MSSA. We performed the assay without any sample preparation and using 50 µl of each sample.
Results: We obtained a complete concordance for 20 samples out of the 27. For 4 samples (3 respiratory samples and 1 cerebral abcess), no data were obtained (error system). But a previous treatment of these 3 respiratory samples which were very viscous with Digest-EUR® (Eurobio) and a mechanical lysis with the Fast Prep® (MP Biomedicals) for the cerebral abcess allowed us to obtained a result. Finally, we obtained discordances for only 4 samples, 1 serum, 1 cutaneous abcess, 2 respiratory samples. For the serum and abcess, the result was negative for *S. aureus* and MRSA detection. For the respiratory samples, MRSA was detected although the strains isolated were susceptible to meticillin.
Conclusion: This preliminary study is very promising. The GeneXpert Platform with the Xpert SA-MRSA kit could be a very useful tool for the rapid detection not only of *S. aureus* but also of MRSA for critical care patients with severe sepsis. Our results need to be confirmed in a more large study.

Molecular virology

R2153 Identification of hepatitis B virus genotype in patients with hepatocellular carcinoma in Iran

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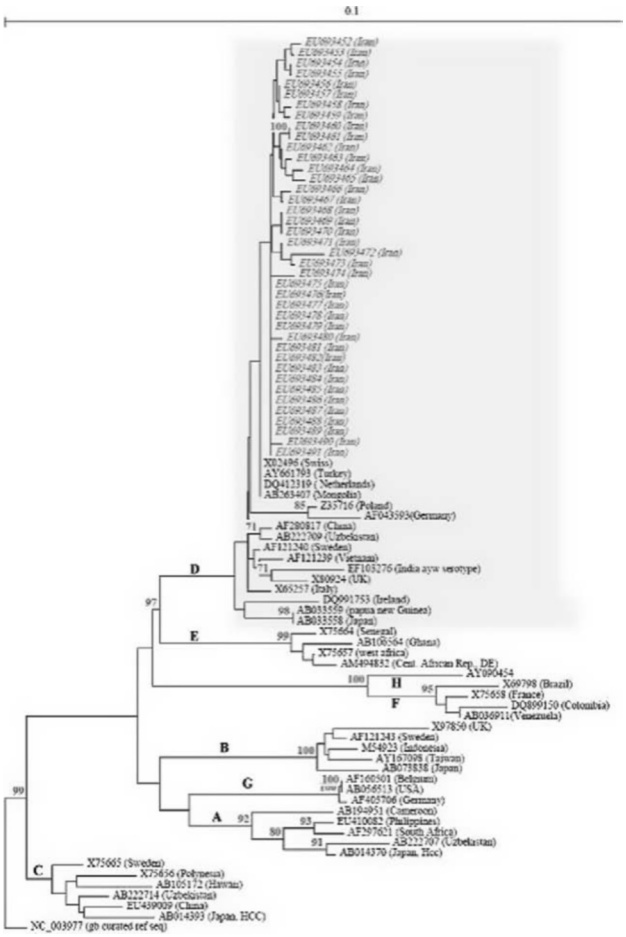
Objectives: Hepatitis B virus (HBV) is one of the major causative agents of hepatocellular carcinoma (HCC) in Iran. The development of HCC in patients with HBV infection has been associated with specific HBV genotypes. The aim of this study was to identify the HBV genotypes in patients with HCC in Iran.
Methods: This study was performed on paraffin-embedded tissue samples of 40 patients (31 males and 9 females) with HBV-associated HCC. HBV genotyping was performed by nested PCR-mediated amplification of the target sequence. PCR products were sequenced, and the genotype of each HBV sequence was determined by comparison with sequences of known genotypes in the GenBank and Phylogenetic tree was constructed.

Results: Phylogenetic analysis revealed that all of the HBV isolates were clustered in the genotype D.
Conclusion: Our results concurred with other reports from Iran all showing that genotype D is the only detectable genotype in different clinical forms of HBV infections in this country.



R2154 New approach for diagnosis of Crimean-Congo haemorrhagic fever in Bulgaria

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Objectives: Crimean-Congo haemorrhagic fever (CCHF) is a tick-borne acute viral disease with leading haemorrhagical syndrome and high fatality rate. The Balkan Peninsula is an endemic region for this disease and sporadic cases or even outbreaks are being observed every year. Diagnosis of CCHF is important for the treatment, outcome of infection and prevention of its further dissemination. The aim of this study is to present results from modification and adaptation of the conventional RT-PCR, nested RT-PCR and Real time RT-PCR system for fast and specific detection of Crimean-Congo Haemorrhagic Fiver Virus (CCHFV) in Bulgaria.
Materials and Methods: Blood samples from patients, suspective for CCHF were drawn, transported and kept frozen in the Vector-borne Infections laboratory at the National Center of Infectious and Parasitic Diseases in Sofia. Also, suspensions with cultivated CCHF virus in mice brains were stored frozen at –70ºC until the examination or were directly used for RNA extraction procedure protocol. Different pairs of oligonucleotide primers, binding to the viral sequence in the region of the small (S) segment of the CCHFV genome were applied.
Results: A single DNA band corresponding to the well known 536 bp PCR product was detected by RT-PCR. Two hundred sixty bp amplicon size was found after the second round of the Nested RT-PCR with different pairs of praimers. A system for faster detection of CCHFV by one step Real time RT-PCR was applied. Positive control reaction was made from the nucleic acid extracted from infected mice brains. Melting curve analysis was used to demonstrate difference between the specific amplification products and non-specific ones.
Conclusion: We introduced various systems of molecular techniques for rapid detection of CCHFV for first time in our country. The combined use of conventional RT-PCR, nested RT-PCR, Real time RT-PCR and serology, will allow differential diagnosis of suspecive patients with viral haemorrhagic fever in the endemic region.

R2155 The prenatal diagnosis for Cytomegalovirus in pregnant women

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Objective: In this study, we aimed to determine the ratio of CMV seroconversion and the presence of CMV DNA in amniotic fluids from pregnant women in the Aydın region of Turkey.
Methods: During the period between March 2006 to December 2008, CMV-specific IgG and IgM were determined by EIA in 100 pregnant women who were performed amniocentesis at 17 to 21 weeks of gestation. Avidity index was studied by EIA. The pregnant women were classified as seropositive or not according to the presence of CMV-IgM and CMV-IgG and a primary or a recurrent CMV infection according to avidity index of anti-CMV IgG. CMV DNA was investigated in the amniotic fluid and blood samples by Real-Time (RT) polymerase chain reaction (PCR) assay.
Results: The rate of seropositivity was found as 95% and seronegativity as 5% in pregnant women and the fetal infection rate was found 1%. The only a pregnant woman was positive for CMV DNA by RT PCR and high avidity index. The infant whom mother determined CMV DNA in her amniotic fluid showed high avidity index. The another pregnant woman was found CMV IgM positive and also high avidity index but not detected CMV DNA in her amniotic fluid.
Conclusion: We concluded that the fetal CMV infection rate is very low due to high maternal seroprevalence in our region.

Molecular typing

R2156 Genotypes of *Helicobacter pylori* isolated from the patients with hepato-biliary diseases

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Objective: To evaluate the incidence of *H. pylori* virulence genes in the liver biopsies and the bile specimens.
Method: 50 patients with chronic cholecystitis and 50 patients with chronic alcoholic cirrhosis were included in our study. The presence of genes *H. pylori* was confirmed by polymerase chain reaction (PCR) using *H. pylori* specific primers for ureC, babA2, cagA, vacA ("Helicopol") according to the recommendations of the manufacturer "Lytech", Moscow (Russia).

Table. Genotypes of *H. pylori* isolated from the bile and liver biopsies

H. pylori genes	n (%)	
	Chronic cholecystitis (N = 27)	Cirrhosis (N = 36)
vacAs1	15 (55.56±9.56%)	15 (41.67±8.22%)
vacAs2	3 (11.11±6.05%)	7 (19.44±6.6%)
vacAs1/s2	2 (7.4±5.04%)	0
vacAm1	0	4 (11.11±5.24%)
vacAm2	14 (51.85±9.66%)	18 (50±8.33%)
vacA–	7 (25.93±8.43%)	14 (38.89±8.13%)
cagA+	12 (44.44±9.56%)	0
cagA–	15 (55.56±9.56%)	36 (100%)
babA2+	8 (29.63±8.79%)	0
babA2–	19 (70.37±8.76%)	36 (100%)

Results: *H. pylori* DNA was isolated from the bile and liver biopsies samples in 54% cases of chronic noncalcular cholecystitis and 72% cases of cirrhosis. Results of *H. pylori* genotyping are presented in the table.
Conclusions: The prevalent genotype of *H. pylori* isolated from the patients with hepato-biliary disorders is UreC positive VacAs1 m2 positive CagA and BabA negative. The received data indicate the possible role of CagA negative *H. pylori* in biliary tract and liver diseases.

R2157 Typing analysis of consecutive *C. krusei* isolates among haemato-oncology patients

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Many reports have documented the emergence of non-*albicans* species of *Candida* as important nosocomial pathogens, like *C. krusei*. The origin of that colonisation/infection can be exogenous, while the primary reservoir in the hospital setting is unknown, it is often linked to a prior colonisation (endogenous). Molecular typing methods, aiming to determine genetic relatedness help to clarify the origin of the strain as well of its main routes of transmission.
Objectives: In order to clarify the hypothesis of an outbreak of *C. krusei* among haemato-oncology patients, mitochondrial DNA typing was used.
Material and Methods: From three hospitalised patients admitted in single rooms at the neutropenic unit of Hospital S. João, Porto, within three weeks, *C. krusei* was isolated from distinct body sites; two of the patients had been admitted consecutively in the same room. All patients had received fluconazole-prophylaxis until the first *C. krusei* isolation. From one of these patients, an additional *C. krusei* strain, isolated from the blood two years before, was included, as well as one *C. krusei* strain obtained from a distinct patient admitted at another department. From both rooms, air and surfaces samples were collected. The identification of the yeast isolates was performed using Vitek 2 system (BioMérieux, Paris) and susceptibility profile to the main antifungals assessed accordingly microdilution CLSI protocol M-27 A2. Total DNA was extracted using a phenol-chlorophorm-isoamilic alcool protocol and quantified; 25–30 μg of DNA were digested with the restriction enzyme, Hinf I, at 37ºC, during 9–12 hours. The digestion reactions were run on 1% agarose gel, for 3–5 h, 120 mV and visualised in UV light. Restriction patterns were compared between strains.
Results: *C. krusei* were recovered from both room surfaces; the air samples were found negative. Considering all the studied isolates, only those recovered from the same patient and its respective room, showed indistinguishable electrophoretic patterns. Isolates from different patients were unrelated. Susceptibility profile was similar for all evaluated strains.
Conclusion: The molecular strategy described, showed a good discriminatory power. It did not support the hypothesis of an outbreak. Moreover, these findings highlight the possibility of an endogenous reservoir, even for long periods of time. This fact should be taken in account on future antifungal therapeutic protocols.

R2158 Molecular typing of *Klebsiella pneumoniae* in neonatal intensive care units using amplified fragment length polymorphism analysis

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Objectives: The aim of the study was to determine the population dynamics of *Klebsiella pneumoniae* in neonatal intensive care units (NICUs) of two major hospitals in Estonia, using amplified fragment length polymorphism (AFLP) analysis.
Methods: From August 2006 until December 2007, repeated perirectal and nasopharyngeal swabs were collected from all neonates admitted to two Estonian NICUs with suspected early onset neonatal sepsis. Mucosal carriage of *K. pneumoniae* was detected in 51 of 278 eligible patients. Finally, 47 of 51 patients were analysed: 38 in unit A (including 5 patients with blood stream infection) and 9 in unit B (plus 1 patient with blood stream infection but without mucosal colonisation). Depending on the length of hospitalisation, the per patient isolate number varied from 1 to 7. Alltogether, 88 perirectal, 52 nasopharyngal, 3 tracheal and 5 blood isolates were used. The AFLP analysis was implemented as described previously1. After restriction enzyme digestion, the fragments were selectively amplified and separated in agarose gel electrophoresis. The fingerprints on gel were analysed using GeneTools program (Syngene). In parallel, 10 samples of 5 patients were typed using pulse-field gel electrophoresis (PFGE) technique.
Results: After the reproducibility analysis of AFLP method, isolates with up to 3-band pattern difference were considered identical. Two predominant *K. pneumoniae* clonal groups (type A in 26 patients over 7 months and five months later, type K in 6 patients over 2 months) were detected in unit A, while in unit B all colonising *K. pneumoniae* strains were different. In all but one subject within individual concordance between nasopharyngeal and perirectal isolates was observed. All five blood stream infections in unit A occurred during the period of type A *K. pneumoniae* colonisation. On AFLP and PFGE analysis, all five invasive isolates were identical to colonising ones. Ampicillin and cefotaxime resistance was detected in 41% and 1.4% of isolates, respectively, but was not reflected on AFLP fingerprints.
Conclusion: AFLP method identified two predominant colonising clonal groups of *K. pneumoniae* in NICU setting A, whereas in setting B all patients had different strain. Vast majority of invasive disease was caused by only type A *K. pneumoniae* infection, but it is not known whether because of possibly higher virulence or as a result of widespread colonisation.

Reference(s)

[1] Van der Zee et al 2003. J. Clin. Microbiol. 41:798–802

R2159 Amplified fragment length polymorphism for high-resolution typing of *Listeria monocytogenes* from foods and the environment

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Objective: *Listeria monocytogenes* is a Gram-positive food-borne pathogen that is commonly present in the environment and occurs naturally in many foods. It is the causative agent of listeriosis, a severe food-borne disease associated with a high case fatality rate. The recent association of *L. monocytogenes* with several large food-borne disease outbreaks suggests that contaminated foods, including meat, dairy, vegetable and fish products, may be the primary source of the organism. Control of food-borne bacterial pathogens is predicated upon the identification of their sources and routes of transmission. Source tracking of *L. monocytogenes* has proved to be difficult as it is ubiquitous in the environment and also because cases are generally sporadic and outbreaks are rare. Recently, several molecular methods such as ribotyping, Pulsed-Field Gel Electrophoresis (PFGE), Amplified Fragment Length Polymorphism (AFLP), multilocus sequence typing have been used to characterise *L. monocytogenes* isolates. In this study we compared two AFLP protocols to characterise 162 *L. monocytogenes* isolates from foods and environmental sources.
Methods: Four selective primer combinations using the restriction enzymes, HindIII and HhaI, and a previously published protocol using EcoRI and MseI, were evaluated for their suitability in genotyping *L. monocytogenes*. Based on the number and distribution of the amplified bands, a protocol using HindIII and HhaI was selected for further genotyping of a collection of 162 *L. monocytogenes* isolates from different food and environmental sources.
Results: On the whole, 28 different AFLP types were identified. The differences in the fingerprinting profiles clearly distinguished two genetic lineages, recognized by serotyping, with a similarity level of 55%. This provided confirmatory evidence that there is a phylogenetic divergence between the strains of serotypes 1/2a, 1/2c, 3a, 3c that formed one cluster and the strains of serotypes 1/2b, 3b, 4b/4e and 4c forming

another cluster. A different genetic variability was observed among the serotypes, with the 1/2a and 1/2b strains exhibiting 13 and 8 AFLP profiles, respectively, out of a total of 75 and 23 analyzed strains. By contrast, all the isolates (n = 30) of the 1/2c serotype were grouped into a single AFLP type.
Conclusion: AFLP fingerprinting using HindIII and HhaI is suitable for high resolution genotyping of *L. monocytogenes* in epidemiological studies.

R2160 Molecular epidemiological analysis of group A streptococci with covR expression at different phases of growth

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Objectives: emm1 type is one of the most frequently isolated emm types from invasive and non-invasive *Streptococcus pyogenes* (group A streptococcus, GAS) infection worldwide. Our study found that all analyzed emm1 strains express the global regulator covR in early-stationary phase and have the better growth activity than strains with covR expression at exponential phase of growth. However, whether the strains with covR expression in early-stationary phase of growth related to specific clonal cluster is unknown.
Methods: Clinical isolates collected from 1994–2003 were analyzed by Northern hybridisation for the covR expression pattern. The multi locus sequence typing and pulsed-field gel electrophoresis typing were used to generate the dendrogram to analyze the clonal relationship of these strains.
Results: All emm1 strains expressed covR at early-stationary phase of growth. Multi locus sequence typing analysis showed all emm1 strains have the identical sequence type (ST28). In addition, pulsed field gel electrophoresis analysis showed emm1 clinical isolates have >75% genetic relatedness. Dendrogram analysis showed only clonal cluster comprises emm1/ST28 strains were expressing covR at early-stationary phase of growth.
Conclusion: These results indicate that the specific covR expression pattern should be a potential determinant phenotype for pandemic predominant emm1/ST28 strains.

R2161 Molecular epidemiology of *Brucella melitensis* in Greece assessed by multilocus variable-number tandem repeat markers

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Brucellosis remains a common infectious disease in many parts of the world, notably in Mediterranean countries and the Middle East. The availability of discriminatory molecular typing tools to inform and assist conventional epidemiological approaches would be invaluable in controlling these infections, but efforts have been hampered by the genetic homogeneity of the genus. Recently, a molecular subtyping system based on the number of an octameric tandem repeat sequence at eight loci in the *Brucella* genome has been developed.
A total of 33 clinical isolates of *Brucella melitensis* biovar 2 representative of 33 patients residing in Greece (1–8 in Northwest Greece, 9–13 in North Greece and 14–33 in Central Greece) were genotyped by 8-locus VNTR system (Hypervariable Octameric Oligonucleotide Finger-Prints, HOOF-Prints).
The discriminatory power of the technique was readily demonstrated by the fact that of the 33 genotyping profiles determined, 32 were unique. Only one pair of strains, isolated in the same family (mother and son), gave identical profiles. The most polymorphic markers, as regard number of alleles and range, were HOOF-Prints 5 and 4, followed by HOOF-Prints 7 and 1. These results indicate that VNTRs used in the HOOF technique could discriminate isolates originating from restricted geographical sources, indicating its potential as an epidemiological tool. Further combined analysis, using more strains, and comparison of our results to the profiles of the existing isolates from European and International data banks will elucidate the *B. melitensis* evolution within our territory and its transmission mechanisms, and enable the improvement of brucellosis surveillance and control programs.

R2162 Transverse microbial differences of the human gut evaluated with terminal restriction fragment length polymorphism

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Objectives: Shifts in microbial composition of the human gut are thought to play a causal role in the development and sustenance of intestinal disorders such as inflammatory bowel disease (IBD). Since adherence of microbes is a prerequisite for disease causation, this study set out to investigate if transverse differences in terminal restriction fragment length polymorphism (T-RFLP) profiles of microbial species exist. T-RFLP allows for culture-independent, molecular fingerprinting of complex microbial communities. Transversal differences were studied between biopsies (the epithelial adherent flora: after removal of the mucus layer) and colonic luminal space (transient flora: mucus adherent plus luminal flora).
Methods: Two biopsy samples were taken from each of five locations in the colon from a healthy patient. Endoscopy was performed for screening purposes in light of a positive family history of colorectal cancer. One biopsy from each location was washed with PBS (leaving the mucus layer intact) and the other with dithiotreitol (DTT, destroys the mucus layer). Biopsy samples (PBS and DTT washed) and the associated supernatants were subjected to T-RFLP analysis by amplification of the 16S rRNA gene with labelled primers 8F-FAM and 926R-NED. Amplimers were digested with HinP1I and MspI and the labelled terminal restriction fragments were separated in an ABI 3130XL sequencer. Cladograms were generated using Pearson correlation and UPGMA clustering in Bionumerics software.
Results: Five out of ten supernatants were inhibited in PCR and were excluded from further analysis. The differently washed biopsies showed a similarity of 89.2%. The PBS supernatants representing luminal microbes clustered closely with the biopsies (85.4%). The DTT supernatants representing mucus associated bacteria showed 75.4% similarity with the biopsies.
Conclusion: In this study, the microbiota of the luminal, mucosal and biopsy samples within one patient were similar. The small differences observed seem largely due to differences in bacterial quantity rather than bacterial composition. This was in agreement with quantitative real-time PCR analysis of these samples. Treatment of biopsy samples with DTT does not seem to influence microbial T-RFLP profiles. Future studies with a larger cohort of healthy patients and inclusion of patients with IBD should shed more light on this possible equilibrium between gut lumen and lining and whether or not it is disturbed in intestinal disease.

Molecular biology, including diagnostics – others

R2163 Diagnosis of the pre-patent *Schistosoma mansoni* infection by polymerase chain reaction in sera of experimentally infected mice

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Objectives: This work aimed at ascertaining the role of polymerase chain reaction (PCR) in detection of *Schistosoma mansoni* (*S. mansoni*) DNA during the pre-patent period in sera of experimentally infected mice. Enzyme linked immuno-sorbent assay (ELISA) was used for detection of circulating antigen (CA) of *S. mansoni* as a screening method for the diagnosis.
Methods: Swiss albino mice were infected with two doses of *S. mansoni* (50 and 100 cercariae/mouse). ELISA and PCR techniques were performed on serum samples collected from the infected mice on the first, second, third and fourth weeks post infection (PI)
Results: The ELISA test was positive in 99.66% of samples and the readings were significantly increased with each of the dose and the duration of the infection. PCR technique was positive with all the examined samples including that was negative by the ELISA test. The minimal detectable amount of *S. mansoni* DNA was 1.16 fg. DNA amplification was not achieved with any of the other helminthes used to evaluate the PCR specificity including *S. haematobium* adult worms.
Conclusion: Both assays have proved their validity as diagnostic tools for *S. mansoni* infection as early as the first week PI However, PCR is a promising technique due to its higher sensitivity, specificity and since it is not affected by the immune status of the hosts. Therefore, PCR is more reliable and must be used as a confirmatory test for ELISA negative cases.

R2164 New real-time PCR based molecular assay for the detection of vanA and vanB genes from rectal swabs

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Objectives: This study evaluated the Xpert vanA/vanB assay (Cepheid, Sunnyvale, CA), a fully-automated PCR-based method for rapid detection of clinically significant genotypes of vancomycin-resistant enterococci (VRE) by rectal swabs, at San Giovanni Battista Hospital in Turin, Italy.
Methods: 136 patients at two Intensive Care Units (ICUs), two Haematology and one Bone Marrow Transplant units were screened using double rectal swabs (Copan, Italy). One swab was used for the molecular test and the other was used to perform the culture reference test. Briefly, swab was immersed in Bile Esculine enrichment Broth (Becton Dickinson, Oxford, UK) and plated on chromID™ VRE (bioMerieux, Basingstoke, UK). Both the agar and the broth were incubated at 37ºC for 24 h. Ten microliters of broth was plated on chromID™ VRE (bioMerieux, Basingstoke, UK). Suspect colonies were subjected to E-test to confirm resistance to vancomycin and teicoplanin and to Genotype *Enterococcus* (Hain LifeScience, Nehren, Germany) to identify genotypes.
Results: A total of 165 rectal swabs were tested using Xpert vanA/vanB assay and the culture methods. Hundred and four (63%) specimens were negative in both methods. Four (2.4%) were positive for both genes in Xpert vanA/vanB and in the culture methods. Three (1.8%) samples were positive for vanA gene in both assays. Fifty-four samples were vanB gene positive in Xpert vanA/vanB Assay (32.7%) while 13 (24.1%) were positive with culture-based methods.
Conclusion: Xpert vanA/vanB assay offers great attributes for the detection of VRE. The system is rapid (<1 h) and therefore particularly useful if testing needs to be performed at any time, day or night. This is an interesting feature since VRE colonisation can be transient and cultural methods require at least 48 hours. Some issues have been highlighted for the vanB gene. If the organism carrying the VanB gene needs to be known a culture-based methods should be used.

R2165 Evaluation of the GeneXpert GBS assay in determining group B streptococcus colonisation

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Objectives: It is recommended that all pregnant women be screened for group B streptococcus carriage at between 35 and 37 weeks of gestation. However intrapartum screening test for GBS could reveal more exact status of GBS colonisation at the time of delivery and would obviate the need for prenatal screening. One of the latest development in the field of the molecular diagnosis of GBS is a fully automated real-time PCR assay, the GeneXpert GBS (GXGBS; Cepheid, Sunnyvale, CA, USA), of which total analysis time is 75 min. In this study, we prospectively evaluated the performance characteristics of GXGBS and in comparison with selective culture in pregnant women. In addition, this study is designed to provide data on the rates of maternal carriage of GBS in Korean women and characteristics of isolated GBS.
Methods: We also performed analytical sensitivity, specificity and precision of GXGBS. We compared GXGBS and standard selective culture with Todd-Hewitt broth in 175 pregnant women at 35–37 week gestation. Specific identification of colonies was done by Vitek-2 and Lancefield grouping. Further serotyping was done using conventional and molecular methods.
Results: For GXGBS, the 95% detection limit was 1.41×10^3 CFU/swab and no cross-reactivity was found. The CVs of within-run, between-run and total precision were 2.8%, 2.6%, and 3.3%, respectively. Detection rate of GBS carriers by GXGBS was 11.4% (20/175), compared with 8.6% (15/175) of selective culture method. The sensitivity, specificity, positive and negative predictive values of the GXGBS compared with selective culture were 86.7, 95.6%, 65.0%, and 98.7%, respectively, The most frequent serotypes were Ia, III, and V (72.2%). All strains were susceptible to penicillin, but a considerable proportion was resistant to erythromycin (44.4%) and clindamycin (44.4%).
Conclusion: GXGBS is a highly sensitive and specific tool for the detection of GBS. Simple preparation and rapid results are attractive points of this assay. GXGBS might be substitutive test for the detection of GBS in antenatal and intrapartum screening test.

R2166 Xylitol and capsular gene expression in *Streptococcus pneumoniae*

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Objectives: Xylitol is a sugar alcohol that inhibits the growth of *Streptococcus pneumoniae*. Xylitol decreases the occurrence of acute otitis media in day-care children but does not decrease the nasopharyngeal carriage of pneumococci. In our previous studies, we found that xylitol changes the ultrastructure of the polysaccharide capsule of pneumococci. We hypothesized that xylitol could affect the expression of pneumococcal capsular genes.
Methods: We investigated capsule gene expression in twenty-four pneumococcal clinical isolates and one ATCC strain (49619) by using a two-step real-time RT-PCR method targeted at the second gene of the pneumococcal capsular locus, the cpsB gene. The pneumococcal isolates were exposed to 5% glucose, 5% xylitol and control medium (brain heart infusion medium supplemented with 10% fetal bovine serum) for two hours. After two hours, bacterial suspensions were treated with RNA-stabilising solution and subjected to RNA extraction and first-strand cDNA synthesis. CpsB gene expression levels were measured by a relative quantification method where we used the housekeeping-gene 16SrRNA of *S. pneumoniae* as a reference gene. The cpsB and 16SrRNA PCRs were performed in separate runs but using the same cDNA sample. The calibrator normalised gene expression levels were determined by using the Relative quantitation tool in the LightCycler software version 4.05. (Roche Diagnostics). Statistical significance was assessed with the analysis of variance for repeated measures.

Results: Exposure to xylitol lowered the cpsB gene expression levels significantly compared to control (P = 0.035) and glucose media (P = 0.011). Capsular gene expression levels in glucose medium did not differ significantly from those measured in control medium (P = 1.000).
Conclusion: We found that xylitol significantly decreased capsular gene expression levels in *S. pneumoniae* isolates compared to control medium. This finding further explains the good clinical efficacy of xylitol in preventing otitis media.

R2167 Prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, herpes simplex virus type 1,2 and papilloma virus in pregnant women in central Greece

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Objective: To examine the prevalence of genital pathogens including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, Herpes simplex virus types 1 and 2 (HSV1, HSV2) and Papilloma virus (HPV) among asymptomatic pregnant women of Central Greece.
Methods: A total of 269 pregnant women during the first trimester of pregnancy, aged from 18 to 42 years old, presented during 2008 at the University Hospital were included. Cervical swabs were obtained and DNA extraction was performed in all specimens. The presence of *C. trachomatis* was examined firstly by direct *C. trachomatis* antigen detection (Cypress Diagnostics) and by Real Time PCR (Nanogen Advanced Diagnostics). DNA of *N. gonorrhoeae* was detected by a home-made PCR, while DNA of HSV1 and HSV2 were detected by Real-Time PCR (Nanogen Advanced Diagnostics). The presence of HPV DNA and oncogenic potential was assessed using PCR followed by a restriction method (Maxim).
Results: Four out 269 women (4.8%) were positive by RT-PCR for *C. trachomatis*; the direct detection of *C. trachomatis* antigen was identified in only one out the four cases. In addition, two women (2.4%) were positive for HPV type 16. DNA of *N. gonorrhoeae*, of HSV1 and HSV2 were not found in any women.
Conclusions: *C. trachomatis* and HPV type 16 were identified in asymptomatic pregnant women in Central Greece. These results support a strategy of screening pregnant women for these pathogens (followed by treatment of infections), which could be integrated into routine pregnancy care in our area.

R2168 Comparison of two commercial molecular tests for routine diagnosis of *C. difficile*

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Objectives: *C. difficile* is currently one of the most important human pathogens and infection incidence and severity have increased significantly over the last five years. The rapid and sensitive diagnostic tests are of crucial importance for correct treatment and for disease control within the hospital environment. However, currently available immunological tests detecting toxins A and B have low sensitivity. New molecular tests could improve sensitivity and enable to detect additional markers such as binary toxin gene and deletion in tcdC gene, both indicating the higher possibility of hypervirulent type 027.
Methods: Routine *C. difficile* diagnosis in our laboratory includes culture (CLO plates, bioMerieux) and direct toxin test (VIDAS CDAB, bioMerieux). In this study two molecular tests, BD GeneOhmTMCdiff Assay (BD Diagnostics, GeneOhm) and Xpert *C. difficile* Assay (Cepheid), are additionally performed on all routine samples.
For BD and Cepheid test swab is briefly immersed in the unformed stool and further processed according to manufacturer instructions. BD test gives result as Positive, Negative or Unresolved, while Cepheid specifies Toxigenic *C. difficile* positive, Toxigenic *C. difficile* negative and 027-NAP1-BI presumptive positive or 027-NAP1-BI presumptive negative.
Results: Approximately 23% of our routine samples are culture positive. CDAB test used for direct detection of toxins is often negative for culture positive samples (also if containing toxigenic *C. difficile* strains) Cepheid assay detected *C. difficile* in all culture positive samples, but some culture negative samples had to be retested due to invalid results and inhibition of PCR reaction. None of the samples was recognized as presumptive 027, which was confirmed by ribotyping of isolated strains. BD had somewhat lower sensitivity but no repetition of tests was needed. None of the molecular tests was positive for *C. difficile* culture negative samples.
Conclusion: Both molecular amplification assays correlated well with toxigenic *C. difficile* culture. Their high specificity and short run time until results (from 50–90 minutes) allows quick and correct diagnosis of *C. difficile*.

R2169 Multiplex real-time PCR for the detection of intestinal protozoa indicates unnecessary treatment of *Entamoeba histolytica* in Turkish patients with intestinal complaints

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Objectives: In Turkey the diagnosis of intestinal parasitic diseases is primarily based on clinical signs and on the epidemiological distribution of the parasite concerned. Laboratory diagnosis is usually limited to microscopy of a direct faecal smear, without further differentiation between the pathogenic *Entamoeba histolytica* and the non-pathogen *E. dispar*. Copro-antigen detection by ELISA is increasingly used, but is still not generally available, while detection of parasite DNA by PCR can only be offered by a limited number of university hospitals. In this pilot study, the diagnostic value of routine microscopy was assessed by comparing its outcome with real-time PCR for the detection of intestinal protozoa. The consequences for clinical management of diarrhoea patients in the region are discussed.
Methods: Seventy-nine stool samples were included in the study. All patients were suspected to have either amoebiasis or giardiasis based on clinical signals and referred for stool examination to the microbiology laboratory of the Ankara Training and Research Hospital. Microscopy of a direct smear from unpreserved stool samples was performed immediately after delivery. Aliquots were sent to the Netherlands for further molecular diagnosis. DNA isolation and multiplex real-time PCR was performed for the specific detection of DNA of *E. histolytica*, *E. dispar*, *Giardia lamblia*, *Cryptosporidium hominis*/*C. parvum*, *Enterocytozoon bieneusi* and *Encephalitozoon* spp. Phocin Herpes Virus 1 (PhHV 1) was added within the isolation lysis buffer to serve as an internal control to detect inhibition factors.
Results: Based on microscopy, 27 (34%) cases of *E. histolytica*/*E. dispar* and 1 (1.3%) case of *G. lamblia* were reported. No helminths were seen. In contrast, real-time PCR detected 2 (2.5%) cases of *E. dispar*, 9 (11.4%) cases of *G. lamblia*, and 1 (1.3%) case of *E. bieneusi*, while no cases with *E. histolytica* or *C. hominis*/*C. parvum* were found.
Conclusion: Although based on limited numbers, our results clearly indicate that direct microscopy in this setting had a low specificity for amoebiasis and a low sensitivity for giardiasis. Unnecessary treatment was given to 27 patients based on the detection of *E. histolytica*/*E. dispar* cysts or trophozoites, while 8 of 9 patients with *G. lamblia* were left untreated. Improvement of microscopy skills, quality control and alternative diagnostic procedures with a higher sensitivity and specificity are recommended.

R2170 Evaluation of the BD Gene-Ohm™ *Clostridium difficile* assay for the diagnosis of *C. difficile* infection

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Introduction: The BD Gene-Ohm™ Cdiff (BD Diagnostics, San Diego, CA) is a real-time PCR assay targeting the toxin B gene (TcdB) of *Clostridium difficile* with a fluorogenic target-specific hybridisation probe for the detection of the amplified DNA. With toxigenic culture (TC) as gold standard, we evaluated the performances of this new assay for the diagnosis of *C. difficile* infections (CDI) on stool specimens

Materials and Methods: Stools were from inpatients of the St Luc-UCL University hospital (890 beds), older than 2 years and suffering from diarrhoea. Cell cytotoxicity (CTA) was performed on MRC5 cells. Cultures were performed on CCFA. In case of positive culture and negative CTA, colonies were tested for 'in vitro' toxin production (TC). Some cultures were repeated on CCFA with added taurocholate (TCCFA).
Real-time PCR was performed according to the manufacturer's instructions.
Results: A total of 749 stool specimens collected in 2008 were tested. Ninety-one samples were shown to contain toxigenic *C. difficile* by CTA and/or toxigenic culture (prevalence: 12.2%). The sensitivity, specificity, PPV and NPV of BD Gene-Ohm™ Cdiff were respectively: 80.2%, 98.2%, 86.5% and 97.1%. Those of CTA were 60.4%, 100%, 100% and 94.8%. Unresolved result was recorded in 43 instances; PCR tests were repeated. Only one result remained unresolved and was excluded from our calculation.
Seventeen false positive cases by PCR were reanalysed by reviewing the patient's records and re-inoculating the stool on TCCFA. In five stools, colonies of toxigenic *C. difficile* were isolated. In two other cases the patient had a history of recent episode of CDI.
Conclusion: The BD Gene-Ohm™ Cdiff demonstrated a very good sensitivity much superior to that of CTA and a good specificity. It is a rapid method allowing result in less than two hours. False positive result may be observed in case of patient with a history of CDI.

R2171 Preliminary evaluation of the Cobas Taqman real-time PCR for *Mycobacterium tuberculosis* detection in clinical specimens

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Objective: We have evaluated the commercially available Cobas Taqman MTB Test, Roche, which is a new released Real Time PCR test for qualitative detection of *Mycobacterium tuberculosis* complex DNA in human respiratory specimens. Our objective was to evaluate the test in both pulmonary and extrapulmonary clinical samples, and to compare the results with those derived from microscopy and our current routine Real Time PCR assay for *M. tuberculosis* (RealArt *M. tuberculosis* TM PCR, Abbott)
Methods: Using the results from Coletsos culture as gold standard we evaluated the sensitivities and the predictive values of the three techniques in 112 samples: 89 respiratory specimens (37 sputum, 28 bronchial aspirates, 14 pleural fluids, 9 bronchial lavages and one protected specimen brush) and 24 nonrespiratory specimens (12 cerebrospinal fluids, 7 exudates and 5 tissue specimens)
Results: The overall results of microscopy, RealArt and Cobas Taqman were: 63%, 85.2% and 81.5% for sensitiviy; 89.5%, 88.5% and 88% for positive predictive value; and 89.4%, 95.4% and 94.3% for negative predictive value, respectively. This last value was higher than 95% for the three techniques in nonrespiratory samples. As described, sensitivities of molecular techniques were considerably lower for smear negative specimens (63.6% and 54.5% for RealArt and Cobas Taqman, respectively)
Conclusions: The Cobas Taqman MTB assay is easy to perform and provides a valuable diagnostic tool for rapid detection of the *M. tuberculosis* directly from clinical samples

R2172 Conventional methods versus PNA FISH for *Candida albicans*/*Candida glabrata* identification

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Objectives: Conventional methods for identification of yeast in blood cultures often take several days. A new peptide nucleic acid fluorescent in situ hybridisation method (PNA FISH), (AdvanDx, Denmark) for yeast identification has a total response time of 2–3 hours. This could be an advantage concerning treatment of patients with invasive fungal infections since they might receive optimal antifungal therapy quicker. We compared how well PNA FISH identification for *C. albicans* or *C. glabrata* performed compared to conventional culture methods.
Methods: Thirty-five blood cultures were positive for *Candida* sp. in our routine laboratory at Rigshospitalet, Denmark during one year from November 2007 to November 2008. *Candida* isolates from 29 (83%) of the positive blood cultures were diagnosed by PNA FISH and also sent to the Danish Mycology Reference Laboratory at the State Serum Institute for culture and confirmatory tests. The conventional methods used were e.g. ChromAgar®, API-strips® and VitekII®.
Results: Thirteen of 29 (45%) of the positive blood cultures were diagnosed by PNA FISH as *C. albicans* and 8/29 (28%) as *C. glabrata*. 8/29 (28%) were PNA FISH negative with growth of other *Candida* spp. than *C. albicans* or *C. glabrata*. In all *C. albicans* and *C. glabrata* cases the PNA FISH results were identical with the results obtained by confirmatory tests performed at the Mycology Reference Laboratory (SSI). The response time for PNA FISH in our routine laboratory was approximately 4 hours and for the conventional methods 3 days, respectively.
Conclusion: We have shown that identification of *C. albicans* or *C. glabrata* by PNA FISH is in agreement with conventional culture methods. By implementing the PNA FISH in the laboratory, the clinical response time for *C. albicans* and *C. glabrata* can be reduced significantly. This could lead to improved patient outcomes.

R2173 Detection of mutations of the tcdC gene of *Clostridium difficile* in stool specimens using a TaqMan-based real-time PCR and melt curve analysis

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Objectives: The tcdC gene product has been proposed to regulate toxin production in *C. difficile* and thus mutations in the gene leading to a truncated or altered TcdC protein may play a role in virulence of the organism. We set out to show that such mutations can be detected directly in stool specimens using a real-time PCR-based method and thus facilitate the rapid detection of virulent strains in clinical specimens.
Methods: DNA was extracted from *C. difficile* strains and clinical stool specimens and subjected to real-time PCR assays. One assay used primers spanning the region of the tcdC gene known to contain the 18 bp deletion. This PCR was designed in such a way that in the subsequent melt curve analysis of the amplicon the presence of the deletion resulted in a higher melting temperature. In the second assay a TaqMan PCR was designed to detect a deletion at position 117 of the tcdC gene, putatively associated with virulence, so that amplification was more than 1000-fold less efficient in the presence of the deletion.
Results: The tcdC-PCR melt curves for strains without the 18 bp deletion showed a mean melting point of 76.86ºC (SD 0.31) and for strains with the 18 bp deletion a mean melting point of 77.26ºC (SD 0.6). The concentration of bacteria and hence DNA had no influence on the results. Using the PCR melt curve analysis we were able to detect the presence or absence of the 18 bp deletion in the tcdC gene in all the *C. difficile* strains tested.
In addition, DNA was extracted from 268 clinical stool specimens and subjected to the real-time PCR assays. Of the 54 specimens positive for *C. difficile* 50 were positive for the PaLoc genes tcdA, tcdB and tcdC, only one of which was positive for the 18 bp deletion. TaqMan PCR detecting the 117 deletion was run on 47 clinical stool specimens two of which were found to contain the deletion. These results were confirmed using the cultured *C. difficile* isolates of the same specimens.
Conclusion: We demonstrate here that the detection of mutations in the PaLoc genes of *C. difficile* can be detected directly in stool specimens. This method is well suited to any laboratory equipped for molecular diagnostics and we envisage development of further applications for the rapid detection of *C. difficile* and possible virulence factors thus facilitating an expedited intensive therapy.

R2174 Role of Toll-like receptors in patients with multiple sclerosis and *Chlamydophila pneumoniae* infection

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Objectives: The possibility that Multiple Sclerosis (MS) is linked to chronic infection by *Chlamydophila pneumoniae* (Cpn) has attracted attention in these last years. Previous studies have demonstrated that in a subset of MS patients with Relapsing-Remitting (RR) forms, Cpn could induce a chronic persistent brain infection acting as a cofactor in the development of the disease. Moreover, mRNA transcript levels of Cpn 16sRNA and HsP60 genes, have been detected in PBMC and CSF compared to other neurological disorders (OND) patients found Reverse Transcriptase (RT) PCR positive for 1 gene only. Literature evidence has established that there is a high expression of Toll like receptors (TLR) in various neurodegenerative disorders including SM (active lesions of microglia and astrocytes). We have evaluated the link between Cpn and the molecular expression of TLRs by studying clinical specimens from MS patients infected by Cpn before and after co-culture on Hep-2 cells specific for this pathogen.
Methods: Fresh CSF and PBMC specimens were obtained from 20 patients with SM RR and 19 controls with OND previously investigated for Cpn 16 s rRNA and Hsp60 genes by PCR and RT-PCR. TLR2 and TLR4 expression was studied by RT-PCR in clinical specimens before and after inoculation and incubation in CO2 for 144 h, on Hep-2 cells.
Results: An evident expression for either TLR2 or TLR4 receptors (9/10, 90%; 7/10, 70%, respectively) was found in patients with SM RR before PBMC but not CSF culture inoculation. These patients have shown to highly express TLR-2 and TLR-4 (6/10; 60%), in contrast with OND patients who were found to express TLR2 (2/10; 20%) or TLR4 (1/10; 10%7) but not both.
Conclusions: TLRs are transmembrane pattern-recognition receptors that initiate signals in response to diverse pathogen-associated molecular patterns. The major expression of TLR-2 and TLR-4 in peripheral blood and not in CSF from SM patients and particularly those with RR forms, indicate that their combined activity might be crucial to modulate and activate cellular-mediated immune response during chronic infections by *Chlamydia*. In fact, MS patients show immunological and cytokine elevations consistent with chronic infections

R2175 Effectiveness of using a new nested PCR tool targeting glmM for *Helicobacter pylori* detection

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Introduction: *Helicobacter pylori* (HP) is a Gram-negative spiral bacterium that causes gastritis, peptic ulcer disease and seems to be involved in gastric cancer. The main aim of the present work was to evaluate the effectiveness of a nested PCR targeting glmM gene by comparing with two other conventional methods, biopsy urease test (BUT) and histological examination (HE). The second objective was to assess the presence of the cytotoxin-associated gene (cagA) in infected HP patients.
Methods: One hundred frozen gastric biopsies from 100 patients were thawed prior to extract the DNA of HP. Nested PCR with a new set of primers targeting the glmM gene was performed, as well as PCR targeting three different segments of the cagA gene. Moreover, HP infection was also established by BUT and HE.
Statistical analyses consisted of a Pearson's chi square test, the association and the correlation among the four variables studied (PCR-glmM, PCR-cagA, BUT, and HE) being determined by Kappa's concordance and Spearman's correlation coefficients.
Results: 88.0% cases were positive for HP by PCR-glmM (52.8% being positives by PCR-cagA), 54.0% by assessing BUT and finally 56.0% were positive by HE. All positives by HE and by BUT were also positive by PCR-glmM.
PCR-glmM was significantly associated ($k = 0.43$, $P < 0.05$) and positively correlated ($r = 0.53$, $P < 0.05$) with HE and with BUT ($k = 0.58$, $P < 0.005$; $r = 0.64$, $P < 0.005$). With regard to PCR-cagA, both concordance and correlation were also found with HE ($k = 0.52$, $P < 0.05$; $r = 0.52$, $P < 0.05$) and with BUT ($k = 0.89$, $P < 0.001$; $r = 0.90$, $P < 0.001$).
The number of glmM positives was significantly higher than the number of cagA ones ($chi = 5.11$, $P < 0.05$), even though all positives by PCR-cagA were also positive by PCR-glmM. Thus, this result confirms the usefulness of nested PCR-glmM and PCR-cagA in detecting, respectively, all and only virulent strains. Knowing the cagA positive HP strains will be useful in predicting the chance for complications as well as for epidemiological and clinical studies of HP infection.
Conclusion: PCR targeting both highly conserved and virulence genes (as glmM or cagA, respectively) is not only concordant with conventional techniques (HE and BUT), but the former method seems to be more sensitive than the latter for detecting HP. For this reason, we suggest that the nested PCR using the new set of primers targeting the glmM gene is a suitable tool to detect HP in clinical samples and it might be included in routine clinical practice.

R2176 A fluorescence in situ hybridisation method using a peptide nucleic acid probe for the identification of *Salmonella* spp. in clinical samples

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Objectives: The diagnostic method currently used for *Salmonella* detection relies on time-consuming plating procedures. However, a tool to assist disease control management and reduce salmonellosis should detect the microorganism in a rapid and reliable manner. In order to achieve this, a fluorescence in situ hybridisation (FISH) method for the rapid detection of *Salmonella* spp. using a novel peptide nucleic acid (PNA) probe was developed.
Methods: The new probe for the specific identification of *Salmonella* was designed, checked against online sequence databases and tested on pure cultures of *Salmonella* and related species. Subsequently, the PNA-FISH method was adapted not only to the detection in artificially contaminated samples, such as blood and powdered infant formula (PIF), but also to multispecies/natural samples (faeces and water). All samples were previously enriched in a rich medium (8 hours for PIF and overnight for the other samples) and analyzed by both PNA-FISH and culture-based methods.
Results: Specificity and sensitivity probe matching theoretical estimates were both 100%. Laboratory testing on representative strains from the *Salmonella* genus subspecies and several related species, confirmed the predicted theoretical values of specificity and sensitivity. The method has been successfully adapted to detect this bacterium in blood, faeces, water and PIF.
Blood and PIF samples artificially contaminated showed that the method was able to detect 1 CFU per 10 mL of blood and also 1 CFU per 10 g of PIF, by performing a previous enrichment step. The faeces and water samples enriched according to the corresponding ISO methods, showed that the PNA FISH method can detect *Salmonella* immediately after conducting the first enrichment step. Moreover, the probe was able to discriminate the bacterium in a mixed microbial population by counter-staining with 4,6-diamidino-2-phenylindole (DAPI).
Conclusions: This new method is applicable to a broad spectrum of samples, taking less than 20 hours to obtain a diagnosis, except for PIF samples where the analysis takes less than 12 hours. Results also showed that this method represents a time saving of at least 48 hours compared to the culture-based techniques. Moreover, results suggested that a new universal approach, reducing the enrichment step for 8 hours as performed for PIF samples, may be developed allowing the use of the technique in routine diagnosis, for a broad spectrum of clinical samples.

Diagnostic/laboratory methods (other than molecular)

R2177 Mean neutrophil volume: a new automated haematologic parameter for acute infection

E. Papakonstantinou, E. Yfantis, S. Chaniotaki, P. Giannopoulou, I. Grafakos, E. Trikka, C. Manti (Athens, GR)*

Review of peripheral blood smears can yield important diagnostic information through the identification of the morphologic changes characteristically seen in reactive neutrophils during infection. This approach, however, is labour-intensive and time-consuming because it requires manual examination. Furthermore, the results are subjective because they depend on human interpretation, and only a few hundred cells can be analyzed for any given sample. The Coulter LH 780 hematology analyzer (Beckman Coulter, Fullerton, CA) has the ability to measure specific parameters of neutrophil populations like mean and standard deviation (SD) of cell volume (MVI, SDVI), conductivity (MCI, SDCI), and light scatter (MSI, SDSI). These so-called positional parameters (PP) can detect morphologic changes in neutrophil population and can be an additional indicator for diagnosing acute infection.

Objectives: To investigate the value of the neutrophil MVI, generated by VCS technology of the Coulter LH 780 hematology analyzer, as an additional predictor of acute infection.

Methods: Total white blood cell count, percentage of neutrophils, and positional parameters data from 289 patients with positive blood cultures and from 54 age-matched healthy control subjects were prospectively analyzed. We then studied whether changes in MVI correlated with the type of microorganism. Positive cultures were subdivided to Gram(+) or Gram(−) microorganism. The PP was obtained by the Coulter LH 750 hematology analyzer (Beckman Coulter, Fullerton, CA). Comparisons between means were performed by analysis of variance. Comparison between 2 means was performed by using the Student t test. A P value less than 0.05 was considered significant.

Results: See the table.

Table

	Control N = 54	Patients N = 289	P	Gram+ N = 72	Gram− N = 128	P
MVI mean	146.17	163.44	<**0.001**	162.06	166.1	**0.046**
MCI mean	145.85	140.99	<**0.001**	139.46	138.97	0.829
MSI mean	146.06	137.53	<**0.001**	137.22	138.16	0.711

Conclusion: 1) The MVI increases in acute infection, while MSI decreases. 2) Using an MVI cutoff of 150, as in bibliography, seems that MVI is a good predictor of acute infection. 3) The MVI increase correlated significantly both with Gram(+) and Gram(−) microorganisms but it was greater at Gram(−) microorganisms (P = 0.046). (4) As a quantitive parameter, the MVI has potential use as an additional indicator for the diagnosis of acute infection.

R2178 Quantitative determination of neutrophil volume distribution width as a new automated haematologic parameter for acute infection

E. Papakonstantinou, E. Yfantis, S. Chaniotaki, P. Giannopoulou, I. Grafakos, E. Trikka, C. Manti (Athens, GR)*

Review of peripheral blood smears can yield important diagnostic information through the identification of the morphologic changes characteristically seen in reactive neutrophils during infection. This approach, however, is labour-intensive and time-consuming because it requires manual examination. Furthermore, the results are subjective because they depend on human interpretation, and only a few hundred cells can be analyzed for any given sample. The Coulter LH 780 hematology analyzer (Beckman Coulter, Fullerton, CA) has the ability to measure specific parameters of neutrophil populations like mean and standard deviation (SD) of cell volume (MVI, SDVI), conductivity (MCI, SDCI), and light scatter (MSI, SDSI). These so-called positional parameters (PP) can detect morphologic changes in neutrophil population and can be an additional indicator for diagnosing acute infection.

Objectives: To investigate the value of the neutrophil SDVI, generated by VCS technology of the Coulter LH 780 hematology analyzer, as an additional predictor of acute infection.

Methods: Absolute neutrophils count, and SDVI data from 289 patients with positive blood cultures and from 54 age-matched healthy control subjects were prospectively analyzed. We then studied whether changes in SDVI correlated with patients absolute neutrophil counts (less or greater than 6600/μL). The PP was obtained by the Coulter LH 750 hematology analyzer. Comparisons between means were performed by analysis of variance. Comparison between 2 means was performed by using the Student t test. A P value less than 0.05 was considered significant.

Results: A significant increase in the SDVI was observed in the bacteraemic patients compared with the controls (27.86 vs 20.63, P < 0.001). Such increase was observed even in patients with absolute neutrophil counts less than 6600/μL) (25.58 vs 20.63, P < 0.001). The more dramatic increases were seen in patients with neutrophilia (28.75 vs 20.63, P < 0.001).

Table

	Control N = 54	Patients N = 289	<6600 N = 81	>6600 N = 208	P
SDVI mean	20.63	27.86	25.58	28.75	<0.001

Conclusion: 1) The SDVI increases in acute infection. 2) Using an SDVI cutoff of 23, as in bibliography, seems that SDVI is a good predictor of acute infection. 3) The SDVI increase correlated significantly with neutrophilia but was observed even in patients with neutrophil counts less than 6600/μL. 4) As a quantitive parameter, the SDVI has potential use as an additional indicator for diagnosing acute infection.

R2179 Low blood culture bottle filling volumes in a tertiary referral hospital in Oman

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Objective: Filling volumes of blood cultures are critical. 30–40 mL of patient blood have previously been found to be the optimal volume range to rule out low-level bacteraemia. According to recently published sepsis guidelines 20–30 mL of patient blood should be cultured. As part of our six sigma approach to improving healthcare we conducted a two-month audit into the blood culture bottle filling volumes at our institution to assess compliance with the standard filling volumes.

Methods: Weights of anaerobic and aerobic blood culture bottles were recorded in a spreadsheet against the bottle barcode number before circulating them to all wards. On receipt in the laboratory the weight difference for each individual bottle was recorded and expressed as collected blood volume. Laboratory episodes, culture result and patient details were recorded.

Results: 298 paired blood culture bottles contained a mean blood volume of 4.9 mL per each pair.

In total blood cultures for 283 episodes of clinically suspected bacteraemia were obtained with a mean volume of 5.3 mLs blood per episode. 43 sets (14.4%) and 38 (13.3%) episode cultures were culture positive. 18 (6%) blood culture sets and 19 (6.7%) episode cultures represented true bacteraemias.

278 (98.2%) of all episode cultures did not attain the recommended target blood culture volume of 20 mLs. 2 episode cultures attaining the target volume were culture positive compared with 36 episode cultures that did attain smaller volumes.

Conclusions: Our audit revealed high level of non-compliance with the local and international blood culture filling volume standards. The level of non-compliance was so high that the audit was terminated prematurely in order to take corrective action. In our institution nurses collect blood samples including blood cultures. Before re-auditing the blood culture volumes we will implement an intensive educational program for staff carrying out phlebotomy duties.
In our audit we were unable to compare positivity rates for low filling volumes with those culture volumes for which the target volume for the standard had been attained. This audit proved that it is feasible to carry out audits into blood culture volumes using individually weighed bottles. Future work will focus on the comparison of optimally filled bottles with underfilled bottles with regards to culture positivity rates.

Statistics for total paired blood culture bottles and blood cultures obtained for each septic episode

	Total paired blood culture bottles (N = 298)	Septic episodes (N = 283)
<20 mL blood obtained per episode (%)	N/A	278 (98.23)
Paired bottle underfilled below bottle ranges i.e. <6 mL blood (%)	224 (75.17)	N/A
Paired bottle underfilled below optimal bottle ranges i.e. <16 mL blood (%)	290 (97.44)	N/A
Positive cultures	43	38
Positive cultures amongst suboptimal filling volumes	43	36
Positive cultures for blood cultures with >20 mL blood obtained	0	2
Catheter-related bloodstream infections	6	3
Blood culture contamination	19	16
True bacteraemias	18	19
Positivity rate		
Overall blood culture positivity rate, %	14.43	13.43
True bacteraemia rate, %	6.04	6.71

R2180 Determination of the accuracy and optimal cut-off point for ELISA test in the diagnosis of brucellosis in Iran

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Objectives: Brucellosis is a prevalent infectious disease in Iran and finding a reliable diagnostic method is the most challengeable problem. In this study we opted to determine an optimal diagnostic cut-off point for Elisa test in Iranian brucellosis patients.
Methods: We collected 56 confirmed cases of brucellosis (based on results of blood culture and standard agglutination test) from Department of Infectious Diseases, Imam Hospital, Tehran University of Medical Sciences. Furthermore, blood samples from 126 controls including 73 healthy controls and 53 non-brucellosis febrile patients were collected. In all of the cases and controls ELISA IgG and ELISA IgM levels were measured and compared with each other by the means of box plot graph and receiver operating characteristic (ROC) curve. The sensitivity and specificity ELISA IgG and IgM were fixed in different cut-off values and IgG and IgM levels yielding maximal sensitivity plus specificity were selected for determination of optimal cut-off point.
Results: Nineteen patients had positive blood culture for *Brucella melitensis*. The standard agglutination test results were 1:160 or more in 54 patients. The box plot graph indicated the high degree of dispersion for IgG and IgM data in brucellosis patients compared with non-brucellosis febrile patients and healthy controls. We observed partially overlapping for IgM but not for IgG levels between cases and controls. The area under ROC curve for discrimination between cases and healthy controls were 0.978 and 0.854 for ELISA IgG and IgM, respectively which were significantly different from 0.5 ($P < 0.001$). In this manner, the area under ROC curve for discrimination between cases and non-brucellosis febrile patients were 0.975 and 0.931 for ELISA IgG and IgM, respectively which again were significantly different from 0.5 ($P < 0.001$). After calculation of sensitivity and specificity of ELISA IgG and IgM in different cut-off values, maximal sensitivity (75%) plus specificity (100%) for ELISA IgG while maximal sensitivity of 95.7% plus specificity of 83.3% for ELISA IgM were observed by cut-off of 50 IU/ml.
Conclusion: The results of present study showed that ELISA IgG is a more accurate test than ELISA IgM in the diagnosis of brucellosis. Using a cut-off of 50 IU/ml could be optimal amount for discrimination patients from controls.

R2181 Predictive model of anaerobic bacteraemias

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Objective: To develop and validate a model for the prediction and diagnosis anaerobic bacteraemias.
Methods: The developing model was created in a urban tertiary care hospital, and the validating model in a urban secondary care hospital.
It's a prospective cohort study with derivation and validation set. The derivation set was collected in the tertiary care hospital during 1986, and 1996 (984 episodes of bacteraemia), and the validation set was collected in the secondary care hospital in 2005–2006 (320 bacteraemias).
Results: The derivation set was studied analysing 938 true aerobic bacteraemias in front of 46 true anaerobic bacteraemias. Independent multivariate predictors of true anaerobic bacteraemia were used to develop a model stratifying patients with puntuations of 0 to 11 points, and they were: unknown bacteraemia focus OR 1.13 (IC 1.13–10.54) 3 points (p); abdominal, and skin focus OR 14.85 (IC 6.37–34.62) 6p; hypotension OR 1.99 (IC 0.98–4.04) 2p; absence of vascular manipulations OR 2.62 (IC 1.04–6.60) 2p; age between 40–60 years OR 1.60 (IC 0.51–5.04) 1p; and age over 60 years OR 3.21 (IC 1.19–8.67) 3p. In the derivation set's group with 6 or more points there are 331 aerobic bacteraemias and 40 (12%) of them are by anaerobic bacteraemias. These 40 bacteraemias are the 87% of all the anaerobic bacteraemias diagnosticated in the derivation set group. The positive predictive value with more than six points is of 13%, with a sensibility (S) of 80.4%.
The validation set was studied analysing 320 bacteraemias. The 83.6% (IC95% 71.19%-92.23%) of anaerobic bacteraemias have more than 7 points, and 72.7% have more than 9 points. S:83.6%, E: 73.6%.
The 26.4% (IC95% 21.2%-32.15%) of aerobic and facultative anaerobic bacteraemias have more than 7 points, and only the 11.7% have 9 or more points. The difference of anaerobic bacteraemias with more than 7 points and the aerobic and aerobic and facultative anaerobic ones has a significance <0.05.
Conclusion: Abdominal, and skin focus OR 14.85; hypotension OR 1.99; absence of vascular manipulations OR 2.62; age between 40–60 years OR 1.60 and age over 60 years let us to make a predictive clinical model of probability of anaerobic bacteraemia with a high sensibility and especificity.

R2182 Isolation and identification of *Ureaplasma urealyticum* and *Mycoplasma hominis*: comparison of two methods

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Objectives: *Ureaplasma urealyticum* (*U. urealyticum*) and *Mycoplasma hominis* (*M. hominis*) have been associated with a plethora of clinical manifestations on the genital system of women. The purpose of our study was to compare two methods of isolation of these two mycoplasmas from the vaginal secretions of symptomatic women.
Methods: Vaginal samples from symptomatic women presenting to the Outpatient Clinic of Aretaieion Hospital were studied. We included in the present study 168 samples from an equal number of women positive for *U. urealyticum* and *M. hominis* which were isolated by two different methods. One was the commercial kit *Mycoplasma* IST2 (BioMerieux, France) and the other *Mycoplasma* HUF (Bioprepare G. Papanikolaou S. Sofou, Gerakas, Attika, Greece). After inoculation onto the respective nutrient medium the samples were incubated at 36–370C in aerobic conditions and checked at 24 h and 48 h.
Results: Out of the 168 samples, at 24 h 122 (72.6%) *U. urealyticum* were identified by *Mycoplasma* IST2 with 54 (32.1%) of them being in a concentration of $>10^4$ CFU/ml while 124 (73.8%) *U. urealyticum* were positive by *Mycoplasma* HUF and 48 (28.6%) of these were in a concentration of $>10^4$ CFU/ml. At 48 h 138 (82.1%) *U. urealyticum* were

identified by *Mycoplasma* IST2 and 136 (81.0%) were in a concentration of $>10^4$ CFU/ml while 138 (82.1%) *U. urealyticum* were identified by *Mycoplasma* HUF and 120 (71.4%) were in a concentration of $>10^4$ CFU/ml. On the other hand, at 24 h with both methods were identified 24 (14.3%) *M. hominis*. Four (2.4%) of these were isolated in a concentration of $>10^4$ CFU/ml by *Mycoplasma* IST2 and 18 (10.7%) with *Mycoplasma* HUF. At 48 h by both methods were isolated 30 (17.9%) *M. hominis* out of which 18 (10.7%) were in a concentration of $>10^4$ CFU/ml by *Mycoplasma* IST2 and 26 (15.5%) by *Mycoplasma* HUF.

Conclusions: Both methods proved to be sensitive in the isolation and identification of *U. urealyticum* and *M. hominis*. However, taking into consideration the quantitative detection more *U. urealyticum* were isolated using the *Mycoplasma* IST2 method and, on the contrary, more *M. hominis* with the aid of *Mycoplasma* HUF.

R2183 Performance characteristics of an anti-Typhi Vi IgG ELISA

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Objective: To investigate performance characteristics of the VaccZyme™ anti-Typhi Vi IgG EIA with respect to reproducibility, linearity and normal incidence.

Background: *Salmonella* typhi is a Gram-negative bacterium which causes the systemic febrile illness typhoid fever. The disease can be fatal if untreated killing around 10% of the people infected. Vaccination with the purified Vi capsular polysaccharide can protect adults and children >18 months against typhoid fever. A patient's specific antibody response to the vaccine may be evaluated by the serological determination of their IgG anti-Typhi Vi antibody levels pre- and post-vaccination. Patients with suspected immunodeficiency may be investigated by assessing their ability to respond to specific polysaccharide antigens like those present in the Typhi Vi vaccine.

Method: Anti-Typhi Vi antibodies were determined using the VaccZyme™ Anti-Typhi Vi IgG EIA kit (The Binding Site, UK). The incidence of antibody was assessed in 200 normal UK blood donors of unknown immune status. Intra-assay reproducibility was determined on eight samples (20 replicates) and inter-assay precision was tested on eight samples in duplicate, on six separate occasions. Linearity was demonstrated on three high titre sera.

Results: The incidence of anti-Typhi Vi IgG antibody in 200 normal blood donors was shown to not follow a normal distribution (Shapiro-Wilk $p < 0.0001$) with a 95% confidence interval of 34.4–60.3 U/ml.

Performance characteristic	Anti-Typhi Vi IgG
Intra-assay reproducibility (%CV)	3.2–6.3
Inter-assay reproducibility (%CV)	6.1–12.6
Linearity R^2	>0.999
Linearity % recovery	90

Conclusion: The distribution of IgG antibodies to Typhi Vi in the samples tested is illustrative of a population where the disease is not endemic and vaccination is given to targeted individuals only. The VaccZyme™ anti-Typhi Vi IgG assay demonstrates excellent linearity and reproducibility. By testing pre and post-immunisation paired sera the assay will be of value to aid diagnosis of individuals suspected of immunodeficiency.

R2184 Evaluation of different immunoassays on Liaison®

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Objectives: Our aim was to evaluate different serological assays on the Liaison® (DiaSorin), a fully automated analyser based on chemiluminescence technology, by comparing them with our routinely used methods for antibody detection against cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), varicella zoster virus (VZV) and *Borrelia burgdorferi* (ELISA on BEP 2000 Advance®, Siemens); rubella virus and *Toxoplasma gondii* (Access®, Beckman Coulter) and *Treponema pallidum* (INNO-LIA™ syphilis kit, Innogenetics).

Methods: DiaSorin controls were used to determine within-run imprecision (CV%). Samples classified as positive by routinely used methods mentioned above, were used: CMV (31 IgM; 28 IgG), EBV (28 IgM; 27 IgG), HSV (51 IgG), VZV (24 IgG), *B. burgdorferi* (10 IgM; 37 IgG), rubella virus (49 IgM; 38 IgG), *T. gondii* (17 IgM; 12 IgG) and *T. pallidum* (total Ig 56). Also a number of negative routine samples were analysed. Discordant results were retested with both methods and/or compared with a third method (VIDAS®, bioMerieux or Western blot).

Results: CV's were acceptable and comparable to those claimed by DiaSorin ranging from 5.6%-20.6%.

For CMV, EBV, HSV, VZV, rubella virus and *T. gondii* IgG assays agreement ranged from 90 to 100%. *B. burgdorferi* IgG agreement was 86%; 5 samples were found negative on Liaison® while earlier positive in ELISA. For *T. pallidum* total Ig, all 56 INNO-LIA™ positive samples were detected by Liaison®. The 10 ELISA positive CMV IgM's negative on Liaison® were confirmed as negative by VIDAS®. All ELISA positive samples for EBV IgM screened positive on Liaison®. Out of 20 EBV IgM negative samples, 3 were equivocal on Liaison®. All *B. burgdorferi* IgM ELISA positives were positive on Liaison®; out of 37 IgM negative samples 6 were positive or equivocal on Liaison®. Out of 17 *T. gondii* IgM positives 6 were negative on Liaison® and also on VIDAS®. Out of 49 rubella virus IgM samples tested positive by Access®, 34 yielded a negative result on Liaison® of which only 5 were confirmed positive by VIDAS®.

Conclusion: Most of the evaluated IgG assays on Liaison® showed a good concordance with our routine methods. Further analyses will be done to draw a conclusion on sensitivity and specificity for IgM assays. In our opinion, the Liaison® offers a reliable and easy method for the evaluated serological assays with a high grade of automation, short turn around times and the ability to run up to 15 different immunoassays at a time.

R2185 Evaluation of the IMMULITE 2000 Syphilis Screen Assay in comparison with *Treponema pallidum* particle agglutination

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Objectives: In this study we compared the IMMULITE 2000 Syphilis screen assay with the *Treponema pallidum* particle agglutination (TPPA).

Methods: Totally 322 sera, in which, 150 sera derived from women who were in their third month of pregnancy, 48 sera with a known reactive TPPA (Fujirebio®) result and 124 ad random sera asked for syphilis serology testing. IMMULITE 2000 Syphilis screen assay is a solid phase, one-step chemoluminescent enzyme immunoassay. The solid phase (beads) is coated with purified recombinant *Treponema pallidum* (Tp 17) antigen. The assays were performed on the IMMULITE 2000 instrument (Siemens®)

Results: The agreement between the two test methods was 98% (315/322). The sensitivity, specificity, negative (NPV) and positive predictive values (PPV) were 94%, 100%, 98%, and 98%, respectively. The resolved results using FTA-abs to confirm TPPA are given in table 1. The sensitivity, specificity NPV and PPV were all 100%.

IMMULITE 2000	*Treponema pallidum* particle agglutination (TPPA)			
	Positive	Indeterminate	Negative	Total
Positive	44	1*	0	45
Indeterminate	1*	0	0	1
Negative	3**	3**	270	276
Total	48	4	270	322

*FTA-abs: positive; **FTA-abs: negative.

Conclusion: The agreement, sensitivity, specificity, negative and positive predictive values are comparable with the TPPA.
The confirmed reactive results in both screening methods showed less false positives in the IMMULITE 2000 Syphilis screen assay.
The mostly manually performed TPPA can be replaced by a fully automated system.

R2186 Evaluation of the new Bio-Rad Platelia™ Toxo kits for the detection of toxoplasmic IgM, IgG and IgG avidity

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Bio-Rad has revisited his current offer for Toxoplasmosis in order to improve the practicability and performance to current assays. The new Platelia™ Toxoplasmosis assays (IgG, IgM and IgG avidity assays) is offering full-automation with cancellation of pre-wash step, new sample dilution, ready to use TMB substrate, standardised washing solution, improvement of conjugate preparation and stability.
Objectives: The aim of this study was to compare the results obtained with these new versions of Platelia TM TOXO kits versus the former one for the detection of anti-*Toxoplasma gondii* IgM or IgG and the determination of IgG avidity, in a retrospective study in the routine conditions of our laboratory.
Methods: Previous and new versions of the kits were used strictly following manufacturer's recommendations on EVOLIS™ automated system.
Material: 2 to 3 successive samples from 49 acute toxoplasmosis (AT) (100 samples); 100 cases from chronic toxoplasmosis (CT). CT samples were divided into one group with both IgM and IgG positive (CT-Mpos) and one group with IgG positive and IgM negative (CT-Mneg).
Results: In all groups, IgG were 100% concordant, and IgM 95.4%. Among the ten IgM discordant cases, 6 belonged to 3 AT patients and allowed earlier detection of seroconversion. The 4 other sera were from CT-Mneg group and the detected IgM were considered as long-lasting toxoplasmic IgM. IgG avidity was concordant in 95.7%. Among the 8 discordant cases, 3 of them belonged to CT-Mneg group which shifted from high to intermediate avidity, they were immunocompromised patients. The 5 remaining discordant cases belonged to AT (avidity stepped from low to intermediate) and CT-Mpos groups (avidity stepped from high to intermediate). One of them, the second sample of the AT case, was considered as an already subacute toxoplasmosis, and the two others discordant sera from CT-Mpos were considered as still subacute toxoplasmosis, 6–12 months post-primary infection; finally, the 2 remaining discordant cases were determined as at least 2 years old CT.
Conclusions: The new Platelia™ TOXO kits are user-friendly; IgM detection is greatly improved; IgG avidity determination is generally more accurate and brings to indeterminate section, patients with subacute toxoplasmosis or uncertain toxoplasmic status such as immunocompromised patients.

R2187 Comparative analysis of simulated candidaemia by using 2 different blood culture systems and rapid identification of *Candida albicans*

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Objectives: To determine the time to detection of *Candida* species isolated from the 2 most common automated blood culture systems and to evaluate rapid and widely available methods for presumptive identification of *Candida albicans* (*C. albicans*).
Methods: Simulated candidaemia models of 8 commonly detected *Candida* species were prepared by ATCC standards. The time to detection was evaluated with BACTEC 9240 (Becton Dickinson) and BacT/Alert 3D (bioMerieux). The presence of pseudohyphae clusters by Gram stain and wet preparation was examined. The germ tube test was performed directly from the blood culture bottles. All samples were cultured on blood agar plates and macroscopically examined for the presence of an irregular margin (spiking).
Results: Most *Candida* species (6/8) except *C. glabrata* and *C. krusei* grew more rapidly, which were detected in aerobic bottles than in anaerobic bottles. The clusters of pseudohyphae were observed in cultures of *C. albicans* and *C. tropicalis*. All culture bottles that detected *C. albicans* showed positive results for germ tube tests and macroscopically showed a "spiking".
Conclusion: Aerobic and anaerobic blood culture systems can effectively detect candidaemia. In addition, the direct germ tube test may be the most useful morphological presumptive identification method for *C. albicans*.

R2188 Assessment of sputum Gram stain and culture for *S. pneumoniae* and *Haemophilus* spp. in the aetiologic diagnosis of lower respiratory tract infections in the European GRACE study

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Objectives: To evaluate the usefulness and yield of sputum Gram stain and culture when applied routinely in the aetiologic diagnosis of Lower Respiratory Tract Infections (LRTI) in the European GRACE study.
Methods: From October 2007 through May 2008, a total of 711 adult patients with LRTI in the community were included during the first winter period in a 3 year prospective study in 11 primary care networks in 8 European countries. Among other respiratory samples, sputum specimens were collected before possible antibiotic therapy was started for the aetiologic diagnosis. Specimens were sent to the local laboratory and processed immediately. Specimens were scored according to the number of leucocytes (WBC) and squamous epithelial cells: specimens with ratios of WBC/epithelial cells >1 were defined as good quality sputa, ratios =1 were considered microscopically valid. Gram Stain and culture were performed according to a standardised protocol. Culture was considered diagnostic when *S. pneumoniae* or *Haemophilus* spp were isolated as a predominant microorganism.
Results: Of the 711 patients included, 538 (75.7%) of patients were able to produce a sputum sample: 254 (47.2%) of these were of good quality. 139 (25.8%) of sputa were microscopically valid with equal numbers of WBC and epithelial cells; 27% of sputa contained more epithelial cells than WBC and were considered salivary contamination. A total of 117/538 (21.7%) of sputa were culture positive for *S. pneumoniae* and *Haemophilus* spp. In good quality sputa, culture positivity increased to 84/254 (33.0%): 80 (14.9%) of sputa were culture positive for *Haemophilus* spp, 37 (6.9%) were positive for *S. pneumoniae*. Analysing the results in correlation with quality showed that 59/80 (73.8%) of *Haemophilus* spp and 25/37 (67.6%) all *S. pneumoniae* positives were from good quality sputa respectively; another 12/80 (15.0%) and 6/37 (16.2%) were isolated, respectively, from sputa with a ratio of WBC/epithelial cells =1.
A predominant morphotype in Gram stain was observed in 53/80 of *Haemophilus* spp and in 33/37 of *S. pneumoniae* positive sputa resulting in sensitivities of 66.3% and 89.2% respectively.
Conclusion: In this primary care setting a good quality sputum sample can be obtained from a considerable number of patients who present with LRTI and culture of these specimens had a good diagnostic yield. Gram stain is more sensitive for the detection of pneumococcal LRTI compared to the detection of *Haemophilus* spp.

R2189 Usefulness of chromoID VRE agar for the detection of vancomycin-resistant enterococci in faecal specimen

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Objectives: Rapid and accurate screening methods are required to effectively control spread of vancomycin-resistant enterococci (VRE) and for the successful management of colonised or infected patients. However, conventional culture methods take long time for detection of VRE. We compared the performance of chromoID VRE agar (bioMérieux, Marcy-l'Etoile, France) designed to recover and identify

VRE from clinical specimens with conventional blood agar plate (BAP) based culture.
Methods: From 1, December, 2007 to 31, January, 2008, rectal swab specimens were received for the detection of VRE and they were inoculated blood agar plate and suspicious VRE isolates subcultured and confirmed by Vitek 2 (bioMérieux, Marcy-l'Etoile, France). From 1, February 2008 to 30 April 2008, surveillance VRE screening cultures performed directly chromoID VRE agar (bioMérieux, Marcy-l'Etoile, France) admitted new patients in intensive care unit (ICU). No suspicious growths of chromoID VRE agar plate reported No-VRE. Suspicious growths of chromoID VRE agar plate confirmed by Vitek 2.
Results: 127 specimens from 31 patients were confirmed VRE by conventional blood agar methods. 153 specimens from 31 patients were confirmed VRE by chromoID VRE agar methods. 202 specimens from 202 patients were confirmed non-VRE by chromoID VRE agar methods. The average time for reporting VRE positive specimen was 5days 23hours 50minutes by conventional methods. The average time for reporting VRE positive specimen was 3days 6minutes by chromoID VRE agar methods. The average time for reporting VRE negative specimen was 1days 7hours 28minutes by chromoID VRE agar methods.
Conclusions: ChromoID VRE agar methods revealed the shortest time for reporting VRE and non-VRE. ChromoID VRE agar proved to be a sensitive, specific and rapid method of detection VRE for VRE surveillance.

R2190 Evaluation of Robobact system for isolation of *Salmonella* sp. in faecal samples

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Objective: To validate a Robobact System for isolation of *Salmonella* sp in faecal samples compare to conventional methods.
Introduction: The development of new methods to improve the automation of the Microbiology laboratory seems necessary considering the present low disponibility of qualified human resources.
Methods: 630 samples of liquid or semi liquid faeces have been studied and compared in our unit both by 1. conventional methods, agar *Salmonella–Shigella* – SS – and Hektoen direct culture and subculture of selenite broth after 24 hours of incubation, and 2. Robobact system with SS and Hektoen media following manufacturer's protocol. Robobact system is an automatic sewing method after a 6 hour pre-incubation of the sample in selenite broth at 37ºC. The preliminary identification of colonies *Salmonella*-compatibles was done using enterotube (Becton Dickinson) or the agglutination of antiserum (Oxoid) with final identification and sensibility study using MicroScan Walkaway (Siemens).
Results: 613 cases have been evaluable (including the results of both methods) recovering a total of 54 isolates (8.8%), 51 (8.3%) using Robobact, and 49 (8.0%) using the conventional method, obtaining an excellent correlation between both methods (Kappa coefficient 0.913). The performance of the culture media of Robobact was of 47 (7.7%) isolated cases detected in the SS agar and 42 (6.9%) in Hektoen agar. In 17.6% of the samples processed by Robobact in SS and in a 15.3% in Hektoen agar no growth was detected, not isolating *Salmonella* by the conventional method.
Conclusion: We have obtained the best sensibility using two culture media (SS and Hecktoen). Robobact system may simplify the observation of cultures due to the pre-incubation time before sowing, taking into account that more than a 15% of the samples didn't show any growth without detecting *Salmonella* sp by the conventional method. Furthermore, Robobact has decreased working and response time. All together these results show that Robobact is a valid method for *Salmonella* sp isolation in our microbiology laboratory.

R2191 Efficient antibody panel for the correct diagnosis of Epstein-Barr virus

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Objectives: Epstein-Barr virus (EBV) is responsible for infective mononucleosis, a disease which affects only 5% of young adults in Western countries, as the majority of the population is immune having contracted an asymptomatic EBV infection in early childhood. The aim of this study was to evaluate the most useful diagnostic parameters to confirm or exclude EBV infection in immunocompetent patients with highly suspicious symptoms for the disease
Methods: Between January and June 2008, we received 1336 requests for serodiagnosis of EBV infection by determination of anti-VCA IgG and anti-EBV IgM antibodies; some samples required also determination of anti-EBNA IgG antibodies. The samples came from subjects of all ages, but particularly from young adults. The antibodies were detected using the Liaison® EBV IgM, VCA IgG and EBNA IgG chemiluminescent assays in accordance with the instructions provided by the manufacturer (DiaSorin, Saluggia – Italy).
Results: EBV IgM antibodies were found in 19.4% of the samples, whereas 74.8% were negative; VCA IgG antibodies were positive in 80.9% of the samples and negative in only 19.1%. Of the 167 samples in which anti-EBNA IgG antibodies were determined, 62.9% were positive and 32.3% negative.
Conclusion: The results show the importance of determining anti-EBNA IgG antibodies in the serodiagnosis of EBV infection as the detection of anti-EBV IgM antibodies, alone, may not be sufficient to confirm or exclude it. In our experience, the determination of anti-EBNA antibodies has proved to be very useful tool in discriminating the different phases of EBV infection, allowing us to offer clinicians an extremely efficient parameter to diagnose acute or previous infection.

R2192 Can the nucleic acid amplification test be an alternative to the serologic tests? A prospective study – results of 18,200 blood donors from Turkish Red Crescent

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Aim: Serologic tests having high sensitivity and specificity are used in order to prevent contamination with infectious agents from blood and blood products for transfusion safety.. Present serologic tests have problems like having low sensitivity and weak detection capacity of infectious agents in "window period". We aimed to test the use of NAT (Nucleic Acid Amplification Test) in routine blood screening in Blood Banking.
Method: We used Procleix ultrio (Chiron, USA) test kit based TMA (Transcription Mediated Amplification) for NAT study in serum samples of 18200 donors who were applied to the Turkish Redcrescent between February 2007-September 2008. The NAT positive samples were studied twice. The discrimination of HIV, HCV and HBV of NAT positive samples were performed by Procleix Discrimination (Chiron, USA) test. Otherwise Micro ELISA were used parallely for routine serological screening of Anti-HIV, Anti-HCV and HBsAg with Vironoste HIV Uniform, AG/Ab innotest HCV Ab and Hepanostica ultra HBsAg test kits.
Results: The results of serum samples with serology(+) and NAT(+) (13/18200 and 0.05%) for Anti-HIV, Anti-HCV and HBsAg were detected higher than other NAT studies and we also detected that a transfusion risk can be occurred in every 1400 transfusions.

Table 1. Serologic tests and NAT results

Patterns	Anti HIV/1–2	Anti-HCV	HBsAg
Serology(+) NAT(–)	19	59	17
Sero1ogy(–) NAT(+)	–	2	11
Serology(+) NAT(+)	3	9	297

Conclusion: We suggest that the reason for this higher positive NAT results can be due to donating status of blood donors. Blood donors(almost 100%) of our study were donating the first time. On the other hand, the cost of NAT test are very higher compared with other serologic tests. As a result of we suggest that it is not necessary to apply NAT tests to the all of the blood donors because of the NAT tests are not being cost-effective. We can also advice to apply the NAT tests to the blood donors who will donate the first time.

R2193 Periodontal pathogenic bacteria diagnostic methods for halitosis patients in Latvia

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Objectives: Bad breath is a frequent problem in Latvia and thus for many patients may cause significant patients distress. Oral bacteria hydrolyze proteins and further degrade amino acids, which leads to halitosis. Gram-negative oral anaerobes, such as *Porphyromonas*, *Tannerella*, *Prevotella*, *Treponema spp.*, produce volatile sulphur compounds (VCS) from sulphur-containing amino acids. In advanced cases, significant amounts of the malodorous components produce high levels of volatile sulfhur compounds (VSC), especially hydrogen sulphide and methyl mercaptan, dimethyl sulphide disulphide cysteine, cysteine, methionine, indole, lactic acid and other compounds, which are mostly derived from the affected sites. Preliminary investigation data show that the reason of bad breath or halitosis often is periodontal diseases and periodontal pathogens.
Methods: The study began in 2001 and included 358 untreated halitosis patients (45.4 year, 201 females, 157 males). The periodontal pocket and dorsal part of tongue microflora was analysed by quantitative PRC (micro-IDent®, Hain Lifescience, Germany) for amounts of periodontal pathogenic bacteria: *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythietensis*, *Treponema denticola*, and *Prevotella intermedia*. Periodontal pathogens were analysed before and after periodontal treatment. ANOVA, t-test, and chi-square were used to detect statistically significant differences. Bad breath was confirmed by measurements made by a portable sulphide monitor or halimeter (Interscan Corporation, Model RH-17E USA).
Results: Halimeter measurements of 358 patients showed increased levels of VSC (380 ppb compared with the control group 40–70 ppb). Before periodontal treatment high level of five periodontal pathogens was both in periodontal pockets and dorsal part of tongue. After planed treatment increased level of periodontal pathogenic bacteria was 71.5% ($p < 0.0001$) patients in dorsal part of tongue microflora.
Conclusion: Halitosis can be diagnosed and monitored using different methods, among which halimetric examination is particularly useful. Since there is present protein degradation in the oral cavity and increased amount of VSC we can conclude that there are changes in oral microflora with a large prevalence of oral anaerobes. That explains the frequent incidence of periodontal diseases in Latvia. Data show that halitosis, increased amount of VSC correlate with increased level of periodontal pathogenic bacteria.

R2194 Diagnostic value of procalcitonin in the early detection of the infection and prognostic and evolutive value in sepsis. Comparison with C-reactive protein and leukocyte count

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Objectives: Procalcitonin (PCT) is a precursor of the hormone calcitonin and is produced by the C-cells of the thyroid gland.
Our purpose is to study the utility of the PCT as an acute-phase reactant in the diagnosis of infections of different aetiology in comparison with C-Reactive Protein (CRP) and leukocyte count; and his prognostic and evolutive value in sepsis.
Methods: Clinical and laboratory data of 522 patients (mean age, 65 years) were analyzed. Determinations of serum PCT were performed with a semiquantitative immunochromatografic test (BRAHMS PCT-Q). Test values were <0.5 ng/mL, 0.5–2 ng/mL, 2–10 ng/mL and >10 ng/mL. CRP was determined by an immunoturbidimetric method (CRPLX Tina-quant. Roche) and leukocyte count by an impedance spectroscopy technique (Pentra 120. ABX Diagnostics). The presence of bacterial, viral, parasitic or fungal infections was obtained from routine microbiological analyses. ROC curves were employed to calculate the sensitivity, specificity and cut-off values to predict all kind of infections.
Results: Studying globally all the infectious diseases, we observe that for concentrations higher than 10, the PCT presents a major diagnostic value of bacterial infections with regard to viral, fungal and parasitic infections. These differences are not observed in CRP and leukocyte count values. Analysing our results we verify that inside the bacterial infections, the PCT only would have diagnostic value besides sepsis [AUC:0.82 (0.78–0.87)], in abdominal infections [AUC:0.67 (0.59–0.75)]. We found significantly more cases of mortality in the first 15 days for patients with PCT's values >0.5 (OR = 2.810). We observe a significant decrease of PCT's values in patients treated with antibiotics adapted to their type of infection, which suggests that infection is under control and a good prediction. For PCT's values of 2–10 in the cases of sepsis, a significantly major number of Gram negative bacteria was isolated opposite to Gram positive. Nevertheless, we do not find significant differences between a range of PCT's values and the detection of a certain microbial species. With regard to the risk of mortality we do not observe statistically significant differences between bacteria Gram positive or negative.
Conclusions: The measurement of PCT in serum is better to identify patients with bacterial sepsis than CRP and leukocyte count. PCT value is an useful marker for monitoring the antibiotic treatment.

R2195 Impact of rapid identification of *Staphylococcus aureus* directly from blood cultures using PNA FISH technology

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Objectives: This study compared the performance of the new Rapid peptide nucleic acid fluorescent in situ hybridisation (PNA FISH) assay (AdvanDx) to that of the Standard PNA FISH for identification of *S. aureus* from blood culture bottles positive for Gram-positive cocci in clusters. The rapid assay shortens time to pathogen detection from 3 hrs using the Standard PNA FISH to 2 hrs, expediting prudent therapeutic and infection control decisions.
Methods: Two smears from 33 blood culture bottles newly positive for Gram-positive cocci in clusters were prepared and assayed by both the Standard and the Rapid PNA FISH (AdvanDx, Woburn MA) methods, following manufacturer's instructions. This assay uses colour labeled fluorescent PNA probes targeting specific rRNA sequences; slides are examined with a fluorescent microscope. Smears positive for *S. aureus* appear as bright green cells. Routine culture identification of staphylococci was performed from blood culture media using Staphaurex (Remel, Lenexa KS) and MicroScan (Siemens Healthcare, Tarrytown, NY).
Results: Of the 10 *S. aureus* isolates recovered from the positive blood cultures, 8 were methicillin-susceptible and 2 were methicillin-resistant. The remaining 23 staphylococci were coagulase-negative (CoNS), 48% were *Staphylococcus epidermidis*. The sensitivity, specificity, positive and negative predictive values of both the Rapid and Standard PNA FISH assays for *S. aureus* identification were 100% when compared to routine identification methods. The correlation of the Rapid with the Standard PNA FISH assays was 100%. No false-positive or false-negative results were found.
Conclusions: The Rapid PNA FISH correlates 100% with the Standard assay and can be utilised to rapidly and accurately identify *S. aureus* and differentiate this pathogen from CoNS directly from newly positive blood cultures using inexpensive fluorescent microscopy. This assay is a cost-effective and user friendly alternative to molecular methods for detection of staphylococcal bacteraemia in real-time.

R2196 Correlation between serum levels of ceruloplasmin and acute phase proteins and oxidative stress markers in patients with sepsis

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Background: Acute phase proteins (APP) are elevated in systemic inflammation such as sepsis. Most studied acute phase proteins were: C Reactive Protein (CRP), fibrinogen, Procalcitonin (PCT), Alfa 2 macroglobulin, haptoglobin and alfa 1 acidic glycoprotein. In many inflammatory conditions the markers of oxidative stress are also elevated. Ceruloplasmin is considered both an acute phase and an antioxidant protein.
Objective: this preliminary prospective study compares serum levels of ceruloplasmin to acute phase protein (CRP, fibrinogen, PCT) and oxidative stress markers, such as malonaldehyde (MDA) in patients with sepsis.
Materials and Methods: All patients admitted in Teaching Hospital of Infectious Diseases Cluj-Napoca, Romania, between Jan-June 2008, diagnosed with sepsis according to ACCP/SCCM, were tested in the first day for serum concentration of acute phase proteins and oxidative stress markers. PCR was measured by turbidimetric method, fibrinogen by Claus method, PCT by the semiquantitative immunochromatographic BRAHMS Diagnostica method, CRP by Ravin method and MDA by spectrofluorimetry, using thiobarbituric acid. As statistic analysis we performed ANOVA score and correlation coefficient Pearson.
Results: Twenty patients (13 males), mean age 51.9 years (median 50.5 years). We found high levels of CRP (mean 12.2 mg/dl, CI 8.9–15.5 mg/dl), fibrinogen (mean 679.2 mg/dl, CI 551–807 mg/dl) and PCT (90% of the values were above 0.5 ng/ml). By the contrary, ceruloplasmin levels were lower than normal (mean 22.1, CI 19.7–24.5 mg/dl). MDA levels were high (mean 3.9 nmol/ml, CI 3.3–4.5 nmol/ml). We found a positive correlation between ceruloplasmin and both fibrinogen ($r = 0.7$, $p < 0.01$) and MDA serum concentrations ($r = 0.61$, $p < 0.01$) and no correlation with the other acute phase proteins.
Conclusions: 1. Acute phase proteins and oxidative stress markers are increased in sepsis. 2. We view ceruloplasmin as being an oxidative stress marker rather than an acute phase protein in patients with sepsis.

Methods for antibacterial susceptibility testing

R2197 Direct susceptibility testing of respiratory samples in the guiding of antimicrobial treatment in ventilator-associated pneumonia

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Background: The purpose of this study was to evaluate a direct antimicrobial susceptibility method by E-test, disk diffusion and antibiotic containing media for guiding adequate and rapid antimicrobial therapy in ventilator-associated pneumonia (VAP), compared to standard microbiological procedures (MIC determinations).
Methods: The study was performed in a 21-bed ICU of a University General Hospital in Athens. In a four month period once weekly, bronchial aspirates (BA) were obtained from each hospitalised patient and were streaked directly on MacConkey agar plates containing each of the antibiotics: Ciprofloxacin 2 mg/L, Meropenem 4 mg/L, Piperacilline/Tazobactam 32/4 mg/L, Colistin 4 mg/L and Vancomycin (VAN) 6 mg/L and in two Muller-Hinton agar(MHA) plates. In the MHA plates E-test strips and disks of the above antibiotics and Tigecycline were immediately placed. After 24 hours of incubation, antibiotic susceptibility was determined according to CLSI breakpoints. All samples were also pocessed by standard microbiological methodology.
Results: Among 198 BA that were performed, 166 were positive resulting in 298 isolates (*A. baumannii* 38%, *P. aeruginosa* 24%, *K. pneumoniae* 7.5%, *S. aureus* 5.2%, other Gram(−) 20%). Resistance rates of Gram(−) to carbapenems and colistin were 64% and 16.5% respectively. In total 34% were polymicrobial specimens (80 BA with 2 isolates and 10 BA with 3 isolates) and 62% with quantitative cultures of $\geqslant$10(4). The use of E-test, Disk Diffusion and Antibiotic Containing Media results would have been administrated in the appropriate therapeutic regiment in 76%, 82% and 53% respectively. The sensitivity of Etest and Disk Diffusion method was increased in BA with quantitative cultures of $\geqslant$10(4) independently the number of the isolates. Most discrepancies resulting in inappropriate therapeutic regiment were due to *A. baumannii* and *P. aeruginosa* susceptibility to Colistin and subsequently Meronem and Piperacilline/Tazobactam.
Conclusions: The described tests could serve an early diagnostic tool providing guidance for the initial appropriate therapy in VAP, helping to decrease the overuse of broad spectrum antimicrobial agents.

Public health and community-acquired infections

R2198 High incidence of *Listeria monocytogenes* meningitis in a north-eastern Italian area

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Background: *Listeria monocytogenes* (LM) is a Gram positive rod agent of meningoencephalitis, expecially in immunoompromised patients. Host factors that increase the risk of listeria infection are: pregnancy, acuired immunosuppression, haematological malignancies, diabetes mellitus, renal failure and chronic alcoholism. In northeastern Italy the incidence of listeria infection with cerebral involvement is unknown since it's unsuspected.
Objective: to study the incidence, epidemiologcal and clinical features of meningitis due to LM in a NorthEastern Italian area.
Methods: we have analyzed by means of computed database all the cases of acute meningitis in HIV negative patients admitted to our department of Infectious Diseases between January 1989 and December 2008. Demographic data, predisposing conditions, underlying diseases, CSF cell count, CSF chemical data, CSF bacterial antigens, CSF and blood coltures, clinical therapeutic and outcomes were investigated with an accurate flow-chart. he diagnosis of listeriosis was confirmed by the Centre de Reférences des Listeria of Pasteur Institute in Paris.
Results: during the study period, 183 cases of meningitis have been observed: 91 with purulent CSF and 92 with clear CSF; ratio males/females 1.4; median age 53.5 years patients with purulent CSF and 46.5 years patients with clear CSF (range 16–88). The study was divided infour compaired periods: 1988–1993, 1994–1998, 1999–2003, 2004–2008. The incidence of listeria infection has been increased: from no cases in the first period, 4.4% in the second, 6.7% in the third and 19% in the last period. The incidence for 100,000 persons in the same periods was respectively: none, 0.28, 0.57 and 1.14. Risk factors of the 7 observed cases were: chronic alcoholism, haematological malignancies and advanced age. Symptomatology: fever, confusional state, rigor nucalis, indisposition. In 6/7 cases LCF was purulent with cell count between 700 and 2500/mmc. The last 3 observed cases presented in vitro resistance to ampicillin, so was employed levoflxacin i.v. in association with ceftriaxone. Two patients developped hydrocephalus.
Conclusions: LM is increased in our area and it's related to the global consumption of food. This is an important fact in order to high percentual of isolation (17.4%) in cheese, vegetables and chicken. On th high observed incidence it's indispensable that empirical therapy of acute mininitis contemplate the employ of an active chemioterapic against LM.

R2199 Transmission of *Tannerella forsythia* in man and domestic cats

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Background: The periodontal pathogen *Tannerella forsythia* (Tf) is strongly associated with periodontal bone loss. Carriership in periodontal healthy subjects reaches levels of almost 48% in the Netherlands. The genetic variation of this pathogen is, however, unknown and there are only few data upon associations between clinical status and Tf-genotypes.
Objectives: To obtain more insight in the clonality and transmission of Tf, we developed an amplified fragment length polymorphism technique-analysis (AFLP) and applied them on isolates from related inhibitants (spouses and/or siblings of spouses) of a tea estate on Western Java, Indonesia. In addition, transmission between domestic cats of various age and their owners were studied in a Dutch population.
Methods: AFLP analysis based on restriction enzymes MseI and PstI was developed to observe whole-genome variation of Tf. The AFLP method was optimised to observe sufficient variation and validated on isolates from twenty-seven individual non-linked subjects with periodontitis. In addition, intra- and interexperimental variation was determined. 178 Tf isolates from 72 subjects (one to four isolates per subject) according to 39 married couples were used to determine transmission of the pathogen between couples and/or siblings. These couples were clustered into 17 family trees.
Results: The intra-isolate homology was >96% when a single strain was processed five times in a single experiment. This intra-isolate homology between independent experiments was 78%. Taking this into account, we found that in only 6 out of 72 subjects two AFLP-genotypes were observed. One of the subjects was carrying two genotypes. Each genotype was identical in two separate non-related subjects. Transmission of Tf between spouses or siblings was not observed. Only one subject carried two genotypes that could be recovered in two different unrelated subjects.
From a simulteneous study in the Netherlands it was found that in 50 couples of young domestic cats (<2 yr old) and their owners no identical strains were found. However, in a group of 7 couples of older cats (>5 yrs old) and their owners, one identical strain in a couple was isolated.
Conclusions: In a closed Indonesian population, only 8.3% of the subjects carried more than one Tf-genotype. Transmission between spouses was not found. Identical strains between cats and their owners suggest an environmental source of Tf that might explain the existence of large numbers of genotypes.

R2200 Failure of antirabic postexposure prophylaxis

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Despite of all progresses made in diagnosis and treatment of rabies, prophylaxis remain the most reliable posibilityof salvation of the infected patients. In humans, rabies results almost always in death, the exceptions being known worldwide. The risk of rabies in untreated patients is 50–80% for head and neck region'15–40% for wounds in upper members and 30–40% for legs wounds. Prophylaxis implies several measures specific and nonspecific pre-exposure (reducing the natural reservoir of infection, pre-exposure vaccination) and post-exposure (for rabid animal, for the wounds, post-exposure prophylaxis with vaccine and rabies immune globulin in cases at risk).
Methods: We describe a case of fatal rabies despite of use of Verorab vaccine and equine immunoglobuline. A 40-years old man with history of diabetus mellitus type 2 and alcoholism was admitted in our clinic with fever (39ºC), headache, muscular fasciculations in left arm, fallowed by paresis, left palpebral ptosis (simptomatology started 4 days before admittance). The patient (who worked as a forester) had been attacked by a rabid wolf, 30 days before addmittance.
The attack was violent and resulted in many wounds in left shoulder, neck region, scalp and both legs. Wound cleansing and tetanus toxoid administration were done at a nearby hospital. Because of high risk of bleeding some of the wounds had to be sutured. Post-exposure profylaxis was started in 3 hours after attack, with equine immunoglobulins (Favirab 40 UI/kg) and Verorab (purified rabies vaccine cultured on Vero-cells). Verorab has been administrated on days 0, 3, 7, 14, 21 after exposure, in deltoid region. After the last administration he became feverish and he was admitted in our clinic with described simptomatology.
After admittance patient rapidly deteriorated with dysautonomia, confusion and coma. Although the diagnosis of rabies was obvious, variants of Guillain–Barré syndrome and acute disseminate encephalomyelitis were also considered in view of supervised vaccination profile. The patient died despite mechanical ventilation in 12 days after admittance. Autopsy was positive for rabies antigen.
Conclusions: Failure of rabies post-exposure prophylaxis is extremely rare in our country. A short incubation period, immunodepresion induced by diabetus melitus, failure to infiltrate maximum Favirab locally due to anatomic nonfeasibility and suturing the wounds could have been contributory in our case.

R2201 Influence of probiotics application on occurrence of common colds and flu: results from the Epidemiology of Allergic Disorders in Poland (ECAP) study

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Aims: The influence of probiotics application on occurrence of common colds and flu.
Materials and Methods: The ECRHS II and ISAAC questionnaires were conducted on 2132 persons – 576 in age group 6–7 y.o., 561 in age group 13–14 y.o. and 995 in age group 20–44 y.o. Sample selection: a randomised study based on the PESEL operat, stratified and representative of particular age groups and the two sexes. Respondents are asked about probiotics application and occurrence of common colds, flu and their signs and symptoms for last 6 months.
Results: In age group 6–7 y.o. 88.0% people with common colds or flu applied probiotics and 11.4% did not; 83.3% respondents without common colds or flu applied probiotics and 13.1% did not; OR = 1.215 (95% CI: 0.607–2.438) and p = 0.041. In age group 13–14 y.o. there were respectively 79.5% vs. 19.2% for respondents with common colds or flu and 76.1% vs. 18.6% for without common colds or flu; OR = 1.011 (95% CI: 0.594–1.721) and p = 0.033. In age group 20–44 y.o. there were respectively 56.1% vs. 40.4% for respondents with common colds or flu and 54.7% vs. 41.2% for without common colds or flu; OR = 1.046 (95% CI: 0.73–1.498) and p = 0.926.
Conclusions: Application of probiotics is slightly associated with increased risk of common colds or flu for children, but not for adults. In the case of adolescent p value means significance, but OR is almost equal 1. These results require more studies in order to ascertain, whether increased risk among children is associated with activity of probiotics.

R2202 Adult vaccination rates in Greece

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Objectives: Our study sought to describe vaccination coverage in Greece among the adult population.
Methods: We conducted a random-sampling, telephone based household survey among adult individuals in Greece. For this purpose a sample of 1104 adults representative of the basic demographic, social and geographical characteristics of the overall population of Greece according to the latest national survey, was used. Two target groups were determined for analysis: persons >65 years of age and persons with chronic conditions such as respiratory and heart conditions (other than hypertension), diabetes mellitus and other conditions.

Results: Among adults 25.4% report having been vaccinated after the age of 18. Among the ones that have been vaccinated in adult life, 31% report influenza vaccination, 30% anti-tetanus, 21.9% hepatitis-B vaccination and less than 10% a number of other vaccinations including anti-pneumococcal vaccine.
88% among persons with chronic conditions report having had any type of contact with the National Health System or a private physician within the last three years. Among them, only 20.1% had been recommended to get vaccinated. Vaccination was recommended only to 22.8% of people over 65 years of age and 11% of the overall adult population. Only 3% of persons with respiratory illness, 6.5% of persons with diabetes mellitus, 10% of persons with heart conditions and 7.3% of persons over 55 years of age in respect, were recommended the pneumococcal vaccine by a physician.
Conclusions: Available data show unacceptably low levels of vaccination coverage among vulnerable groups such as the population over 65 years of age and people living with chronic illness. Influenza vaccination is the most common vaccination among all different groups and the main vaccine recommemded by physicians. Other important vaccines recommended to either the general adult population such as the anti-tetanus vaccination or high risk groups such as the pneumococcal vaccine are actually not being recommended by physicians. Focused efforts are required in order to increase recommendation of vaccination by medical services and acceptance by patients in order to succeed in enhancing vaccination coverage specifically among vulnerable groups such as the elderly and people living with chronic illness.

R2203 Brucellosis: a retrospective evaluation of clinical, laboratory and therapeutic features of 101 cases

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Objectives: The aim of this study was to evaluate the clinical and laboratory findings, complications and treatment outcomes of patients with brucellosis.
Methods: One hundred one cases, followed-up in our clinic between the period of January 2001-August 2008, were assessed retrospectively. The diagnosis of brucellosis was established by one of the following criteria; isolation of *Brucella* species in blood or other body fluids or tissue samples, a compatible clinical picture supported by the detection of specific antibodies by standart tube agglutination test (STA) or enzyme-immunoassay.
Results: The patients' mean age was 43.47 years(14–77). Forty-nine patients were male and 52 patients were female. Of them, 44.5% were diagnosed as acute, 47.5% as subacute and 7.9% as chronic brucellosis. Overall, 61.3% of the cases had a history of ingestion of unpasteurised milk and diary products. The most common complaints were fever(72.2%), sweating(57.4%), arthralgia(51.4%) and malaise(50.5%). Lymphadenopathy(17.8%), splenomegaly(34.6%), hepatomegaly(27.7%) were also detected. Leukopenia was determined in 34.6%, thrombocytopenia in 11.8%, anaemia in 47.5%, elevated erytrocyte sedimentation rate in 72.2%, C-reactive protein positivity in 62.3%, and anaemia in 51.7% of them. Blood cultures were performed from 75 of the patients, and 39 (52%) of them yielded *Brucella* spp. The bacterial growth was also detected in a soft tissue abscess and hip joint fluid samples. STA test was found negative in 1.9% of patients who were culture positive. The osteoarticuler involvement was observed in eighteen patients (17.8%). neurobrucellosis in eight cases (7.9%), epididymo-orchitis in two cases (1.9%), endocarditis in two cases (1.9%), hepatitis in two cases (1.9%); and splenic abscess, multiple soft tissue abscesses, mesenteric lymphadenopathies (mimicking acute abdomen syndrom) and arthritis were present in one each. Doxycycline and rifampin combination during six weeks was the most preferred antibiotic regimen. Relapse and unresponsiveness to the therapy were detected in 5.9% and 0.9% of the cases, respectively.
Conclusion: Brucellosis displays diagnostic and therapeutic difficulties because of the various clinical presentations and lead to labour loss and rarely mortality due to serious complications. It should be considered in patients with unexplained signs and symptoms associated with any organ system involvement, especially in endemic areas.

R2204 Investigation of *Toxoplasma gondii* in shellfish from Adriatic Sea, Italy

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Objectives: *Toxoplasma gondii* infections have been reported in a number of marine molluscal bivalve shellfish. How these animals acquire *T. gondii* from their aquatic environment is not known. Some authors have shown that the Eastern oysters (*Crassostrea virginica*) from North America can remove *T. gondii* oocysts from surrounding seawater and retain their infectivity. In order to investigate if *T. gondii* can be acquired from aquatic environment; we searched *T. gondii* by a sensitive PCR in a selected shellfish's set (*Mytilus galloprovincialis*, *Tapes philippinarum*, *Chamaelea gallina*, *Crassostrea gigas*), collected from a restricted tract of Adriatic sea coast (26 km^2) at intensive breeding, to evaluate the possibility that these marine bivalves can assume *T. gondii* oocysts in same way.
Methods: *T. gondii* DNA was investigated by nested PCR on 140 shellfish and seawater specimens. After homogenising, DNA was extracted according to standard protocols and *T. gondii* B1 gene was amplified. All samples also underwent rigorous bacteriological assays, including the search of biotoxins and harmful algae (*Dinophysis* spp., *Alexandrium ostenfeldii*, *Protoceratium reticulatum*, *Lingulodinium polyedrum*) according to European legislation (2004; Reg. EU 853/854).
Results: No sample did detect *T. gondii* DNA. However, bacteriological investigation showed *Salmonella* spp., and *E. coli* in 8 (5.7%) and 11 (7.8%) specimens, respectively, whereas marine biotoxins (7 of Yessotoxin, 13 of Acid Okadaic) were found in 20 specimen (14.3%). No harmful algae were detected.
Conclusions: Although the high rates of either bacteria or marine biotoxins found but not designed for human consumption, the results show that the shellfish are not able to acquire *T. gondii* or retain infectivity for prolonged periods. Consequently, they should not be a possible source of contamination for humans with possible public health implication.

R2205 Comparison of multiple and single liver abscess

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Background: The aim of this study was to compare the difference between multiple and single liver abscess for clinical feature.
Methods: We retrospectively analysed the medical records of 201 patients with a discharge diagnosis of pyogenic liver abscess between January 2004 and July 2006 at a Wonkwang medical centre.
Results: Of the 201 patients, 34 were classified in the multiple liver abscess groups and 167 were classified into the single liver abscess groups. The frequency of underlying biliary disease and multi-septated abscess were higher in the multiple liver abscess group than in the single liver abscess group ($p < 0.05$)). *Klebsiella* pneumonia was most common pathogen (95/138) and *Escherichia coli* was 2nd common pathogen(21/138), but there was no difference between the both groups. The presenting symptom/sign and laboratory finding, underlying disease (except biliary disease) were similar on the both groups. The drainage procedure or medical management alone were used similarly on the both groups. The death rate were no significantly difference between the two groups [3(8.8%) vs. 8(4.8%)].
Conclusion: Our study suggests that underlying biliary disease and multi-septated abscess were associated with multiple liver abscess.

R2206 Asymptomatic bacteriouria in women with diabetes mellitus in our area

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Objectives: Diabetes Mellitus (DM) is a metabolic disorder which is responsible for several abnormalities of the host defence system. Infections and especially urinary tract infections (UTI) are more frequent in patients with DM than the rest population. The aim of this study was to determine the prevalence of asymptomatic bacteriouria (ASB) in women with DM type 2 in our area and to asses the microorganisms isolated of these patients and the antimicrobial sensitivity.
Methods: 287 women with DM type 2 and mean age of 56±17 years were included in this study, as out-patients, during a 18-month period. Patients with symptomatic UTI, hospitalisation or surgery in the last 4 months and use antibiotics for any reason in the last two weeks, were excluded. Demographical data, medical history and duration of diabetes of patients were registered. All patients underwent laboratory examinations (serum creatinin, glycosylated haemoglobin A1c, fasting blood glucose, blood urea nitrogen and urine samples for urianalysis, microscopy and culture). Urine samples were obtained by clean voided mid-stream technique. The criterion used for defining ASB was the presence of at least 10^5 CFU/ml in 1 culture confirmed by a second culture. Mann-Whitney U test, t test, chi squared and Fisher exact test were used for statistical analysis of data.
Results: 42 (14.6%) women with DM type 2 had two positive cultures with the same microorganism. The uropathogens isolated were: *E. coli* in 23(54.8%) cases, *Streptococcus* spp. in 7(16.7%), *Staphylococcus* coagulase negative in 6(14.3%), *Proteus* spp. in 3(7.1%) and *Pseudomonas* spp. in 3(7.1%) cases. The sensitivity of *E. coli*, *Streptococcus* spp., *Staphylococcus* coagulase negative, *Proteus* spp. and *Pseudomonas* spp. to antibiotics was: 69.6%, 85.7%, 100%, 100% and 33.3% to amikacin, 86.9%%, 71.4%, 83.3%, 66.7% and 33.3% to ciprofloxacin, 78.2%, 71.4%, 83.3%, 66.7% and 33.3% to cotrimoxazole, 69.5%, 85.7%, 66.7%, 66.7% and 33.3% to ampicillin, respectively. Duration of DM, level of HbA1c and the presence of albuminuria were not correlated with ASB in our study ($p = 0.1$, $p = 0.45$ and $p = 0.1$ respectively). The age of woman and the presence of glucosuria were significant correlated with ASB ($p < 0.001$ and $p < 0.03$, respectively).
Conclusions: The presence of ASB is quite high in women with DM in our area such as in previous studies. Periodically urine analysis and cultures as a routine examination in diabetes women may help to recognize ASB episodes.

R2207 The effectiveness of different window mesh sizes in the prevention of mosquito–human contact

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Introduction: The use of Insecticide Treated Bednets (ITN) for prevention of mosquito borne diseases has not been fully embraced in Nigeria.
Objective: This study was carried out to assess the efficacy of the two different window mesh sizes as commonly used in Abeokuta, Nigeria. A high proportion of the human residence sampled in the preliminary a survey use only window nets as screen against mosquitoes.
Methods: Newly hatched mosquitoes were kept in specially designed experimental cages for 7 days using window nets of different mesh sizes as screen.
Results: The species of mosquitoes that hatched from larvae collected from different locations in Abeokuta were *Culex quiquefasciatus*, *Aedes aegypti*, *Anopheles gambiae* and *Mansonia africanus* with various anthropometric measurements ranging from head diameter of 0.25 mm-0.50 mm, thoracic width of 0.30 mm-1.05 mm, and abdominal width of 0.30 mm-0.60 mm. None of the mosquitoes was able to pass through any of the two mesh sizes assessed indicating their effectiveness as barrier against mosquitoes of different species. However, 70% of the window nets in the household survey were observed to record various degrees of perforations and damages through deliberate and/or indeliberate activities. Frequency of opening and closing of doors may also contribute additional entry route for mosquitoes.
Conclusions: This study emphasizes the fact that the use of window nets solely may not be sufficient for prevention of mosquito borne infections. There is the need to further encourage the use of ITN in Nigeria.

Emerging infectious diseases

R2208 Multiple cranial nerves involvement caused by *Brucella melitensis*

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Objective: Neurobrucellosis is a rare complication of brucellosis and cranial nerve involvement is rarely observed during the course of neurobrucellosis. We report a case of neurobrucellosis complicated by optic, abducens and vestibulocochlear nerve palsies.
Case report: A 23-year-old woman was referred to our centre for headache starting 20 days ago and gradually exacerbating in severity, nausea and vomiting for the past two weeks and double vision and disturbed balance for the past one week. On admission, the patient had a moderate general wellbeing and was conscious and cooperative. Blood leukocyte count was 3500/mL, erythrocyte sedimentation rate (ESR) was 22 mm/hour and C-reactive protein (CRP) was 0.3 mg/L. Other laboratory investigations revealed normal results. Vital signs were stable. On physical examination, she had neck stiffness, restricted abduction of the left eye (indicative of the left abducens nerve palsy) and bilateral papillary stasis. Other systems of the body were normal.
Lumbar puncture was performed and cerebrospinal fluid (CSF) pressure was 30 cmH2O (normal range:10–18 cmH2O). Microscopic examination of CSF showed 10 lymphocytes/mm^3. Gram and acid fast staining of CSF did not demonstrate any microorganisms. CSF specimens were cultured. CSF glucose was 14 mg/dL (simultaneously measured blood glucose was 90 mg/dL) and CSF protein was 135.8 mg/dL (normal; 15–45 mg/dL). *Brucella melitensis* was isolated in cerebrospinal fluid. The diagnosis of brucellosis was confirmed by serological tests, cerebrospinal fluid biochemistry and serology. The case was diagnosed with retrobulbar-neuritis. Despite medical treatment, the patient developed optic atrophy. Abducens and vestibulocochlear nerve palsies were relieved. It has been reported in the literature that n. abducens and vestibulocochlear nerve involvement frequently occurs, but that that optic nerve involvement is not frequent. It has been noted that optic nerve involvement is frequently accompanied by optic neuritis.
Conclusion: In addition, multiple cranial nerve involvement has not been reported. In conclusion, it should be kept in mind especially in regions where brucellosis is endemic that several cranial nerves may be involved in cases of brucellosis and that differential diagnosis should include brucellosis in patients presenting with blurred vision and double vision.

R2209 Fournier gangrene caused by *Gardnerella vaginalis*: a brief report

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Objectives: Fournier's gangrene is a life-threatening necrotising infection of the perineal and genital regions. If urgent surgery is delayed, the disease will soon result in septic shock, multiorgan failure, and death. *Gardnerella vaginalis* is an infrequent cause of soft tissue infections, bacteraemia and is typically associated with immunocompromised states.
Case: The case presented here refers to an diabetic 61-year-old women, admitted in emergency to our department with clinical signs and symptoms of sepsis related to gangrene of the perineum. The lesion had developed 10 days previously from a carbuncle, which she attempted to treat by way of an over-the-counter products. Lab results were normal apart from the leucocytosis (13700/I) and CRP (6 mg/dl). She was diabetic for the last 11 years. An early wide surgical debridment was performed and empirical intravenous therapy with ampisillin-sulbactam

and ciprofloxacin was initiated. Two days later, results from perioperative swab culture revealed growth of *G. vaginalis*. The patient was released 21 days postoperatively with a clean wound, and no complications.
Conclusion: An examination of the literature revealed no reports of *G. vaginalis* in soft tissue infections such as Fournier gangrene. There are very few reports of infections caused by this pathogen in diabetic patients. As a result, although it is uncommon and the mechanism of infection is unclear, *G. vaginalis* should be included in the causative pathogens in Fournier gangrene.

R2210 The epidemiological, clinical and laboratory evaluation of Crimean-Congo haemorrhagic fever cases in a tertiary-care hospital in Turkey, 2008

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Objective: Crimean-Congo haemorrhagic fever (CCHF) is a serious disease caused by the CCHF virus and has been reported in our country since 2002. In this study we present the epidemiological features, clinical and laboratory findings, treatment, and outcome of cases with CCHF followed in Ankara Training and Research Hospital in 2008.
Methods: Eighty-six patients suspected to have CCHF were included in the study. Serum samples were analyzed with specific ELISA for detecting antibodies (IgM and IgG) against CCHF, and also with RT-PCR to investigate the genome of the virus. Those with positive IgM antibodies and/or PCR for CCHF virus in blood evaluated as confirmed case, and those with negative results as suspected cases.
Results: Among 86 patients, 71 were diagnosed as confirmed cases. Of all the patients, 51 (59.3%) were female; mean age was 48.8±18.2 years (15–83 years). Seventy-four patients (86%) were living in the rural area. Tick bite history was detected in 57 patients (66.3%) and haemorrhage history in 22 (25.6%). Epistaxis and petechia/purpura were the mostly declared haemorrhagic complaints. Mean time from tick bite to admission to the hospital was 8.6±7.2 days (1–35 days). The most common symptoms were fever, fatigue, nausea, myalgia and headache (84.9%, 82.6%, 67.4%, 64.0% and 41.9%, respectively). The mean hospital stay of the patients was 7.3±2.9 days (1–17 days). Median level and minimum and maximum values of some of the laboratory findings were as follows; 2200/mm3 (500–29000) for white blood cells, 57500/mm3 (7000–361000) for platelets, 126 U/L (10–1155) for aspartate aminotransferase, 77 U/L (14–800) for alanine aminotransferase, 216 U/L (19–4564) for creatinine phosphokinase, 10.7 seconds (7.0–109.0) for protrombin time and 37.0 seconds (20.4–105.0) for partial thromboplastin time. Ribavirin and steroid therapies were given to 17 (19.8%) and 22 (25.6%) of the patients, respectively. Among the patients, 44.2% received platelet (random/aferesis), 24.4% fresh frozen plasma and 11.6% erythrocyte infusions. Although supportive therapy was administered, four cases (4.7%) were died because of massive hemorrhage.
Conclusion: In CCHF patients, serum platelet counts, haemoglobin values and protrombin and partial thromboplastin times should be closely monitored. In case who had a deterioration of the related findings, the physicians should consider to adjust supportive therapy.

R2211 Nocardiosis: an emerging disease?

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Introduction and Objective: although *Nocardia* spp. is an unusual pathogen in our settings, its incidence shows a rising trend related to the increase of immunosuppressive factors. The aim of this study is to analyze the clinical and epidemiological characteristics of those cases diagnosed in a 455-bed, University-affiliated hospital in Barcelona, Spain.
Methods: Retrospective analysis (2002–2008) of all patients with nocardiosis consistently documented: isolation from extrapulmonary samples, pulmonary-invasive BAL, or at least two positive-consecutive sputum samples.
Results: During the study period 32 patients (29 men), mean age 73, were included for analysis: 3 in 2002, 4 in 2003, 9 in 2004, 4 in 2005, 4 in 2006, 5 in 2007 and 3 in 2008. The average score on the Charlson comorbidity index was 3.53 (range 1–7). The most frequent underlying disease was COPD (26 patients, 14 of them receiving continuous therapy with steroids), and the main reason for being visited were general, non specific respiratory symptoms. In 14 patients (44%) chest radiography showed lung infiltrates involving alveolar spaces, which improved (or resolved) after antimicrobial therapy. Molecular identification was successfully done in 23 strains: *N. asteroides* 13; *N. cyriacigeorgica* 6; *N. paucivorans* 1, *N. brasiliensis* 1, *N. abscessus* 1; *N. flavorosea* 1. All were sensitive to sulphonamides, aminoglycosides and carbapenems. The empirical therapy most commonly used was cotrimoxazole (21 patients). *Nocardia asteroides* was considered to be the direct cause of death in 8 patients (25%), all with a very severe COPD (FEV1 < 30%), and receiving continous steroid therapy.
Conclusions: The incidence of nocardiosis has slightly increased in our patients, being *N. asteroides* the ultimately fatal lung infection in the most severe COPD cases.

R2212 A case of Crimean-Congo haemorrhagic fever with normal laboratory findings

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Objectives: Crimean-Congo Haemorrhagic Fever (CCHF) is a tickborn disease caused by a Nairovirus of the Bunyaviridae family. Infection is transmitted to humans by Hyalomma ticks or by direct contact with the blood or tissues of infected humans or viraemic livestock. Clinical features usually include a rapid progression characterised by haemorrhage, myalgia and fever, with a mortality rate of up to 30%. Thrombocytopenia, leukopenia, elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatinine phosphokinase (CK) levels are common. In this report, we present a confirmed CCHF case without laboratory abnormality.
Case: A 36-year-old woman admitted to our clinic with the complaints of fever, chills, myalgia and vomiting. She had a history of anaemia for six years. She was living in an endemic region for CCHF and had a history of tick bite ten days ago. Her complaints were started five days after the tick bite and bleeding of the nose and vagen were added subsequently. On the initial examination, body temperature was 37.6ºC, pulse 88 beats/min. No any abnormality was noted on systemic examination except vaginal haemorrhage. The epidemiological and clinical features of the patient were typical for CCHF but the laboratory investigation was normal except anaemia. The leukocyte count was 7300/mm^3, haemoglobin 11.9 g/dL, platelets 293000/mm^3, AST 23 U/L, ALT 14 U/L, ALP 30 U/L, GGT 11 U/L, LDH 139 U/L, CK 39 U/L, INR 0.8 and APTT 26.2 seconds. Vaginal haemorrhage stopped in the third day of the hospitalisation, and haemorrhage from nose one day after. Laboratory values were closely followed-up and no any abnormality was detected. Her symptoms regressed gradually. She had discharged from the hospital on sixth day of the hospitalisation; all the clinical and laboratory findings were normal except anaemia. The diagnosis of CCHF was confirmed with the PCR positivity.
Conclusion: We suggest that patients who were coming from endemic region and who had typical epidemiological and clinical findings should evaluate as a possible case for CCHF even if the laboratory findings were not compatible.

R2213 A case of infective endocarditis caused by *Gemella sanguinis*

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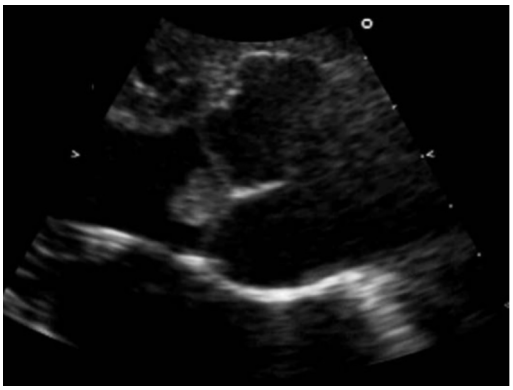
Introduction: Infective Endocarditis (IE) is one of the most commonly recognized infections associated with *Gemella* spp. Approximately 42

cases of IE associated with *Gemella* spp. have been reported in the literature. *Gemella sanguinis* has been reported only in two cases as a cause IE.
Case summary: A 23-year-old male with history of repaired ventricular septal defect and regurgitated aortic valve and history of Behcet's disease who was admitted with the diagnosis of infective arotic valve endocarditis based on a clinical presentation of fever, right flank pain, a new diastolic murmur, mild anaemia, leukocytosis and an elevated erythrocyte sedimentation rate, supported with a positive blood culture for *Gemella sanguinis* and echocardiogram findings of aortic regurgitation with large mass (17×9 mm) that is attached to the right coronary cuspid of aortic valve. An embolisation to the right kidney was confirmed with a computed tomography of the abdomen revealing a wedge shaped infarction. The patient was treated with ceftriaxone and gentamicin and had a good clinical response with resolution of fever, negativity of follow up blood culture and decreased sedimentation rate. The patient had eventually died with intracranial bleed as a complication of anticoagulation that was started for hypercoagulable state associated with Behcet's disease..
Discussion: To the best of our knowledge, this is only the third report of endocarditis caused by *G. sanguinis*. Our patient had a predisposing cardiac disease and in the absence of other predisposing dental or periodontal diseases; we believe that his oral ulcers related to his Behcet's disease served as the entry port of the organism to the blood stream. The vegetation size is relatively lage and associated with visceral embolisation, none of the case reports that described this organism have commented on the vegetations size.
Conclusion: This case represents the first case of *Gemella* IE in the kingdom and the third in the literature.



R2214 *Actinobaculum schaalii*: a new cause of mastitis

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Objective: *Actinobaculum* sp. is a Gram-positive rod closely related to the genus *Actinomyces*. A new species, *Actinobaculum schaalii* was first discovered in 1997 and has been related to urinary tract infections. We report one case of *A. schaalii* isolate found in a breast abscess.
Methods: The drainage obtained was immediately sent to the Microbiological Department. The specimen was cultured in Columbia agar with 5% sheep blood, chocolate agar and *Brucella* blood agar incubated in aerobic, microaerophilic and anaerobic environments, respectively. The strain was characterised by using the Rapid ID32A systems (bioMérieux, France) in according to manufacter's instructions. For definite confirmation, isolate was referred to a reference laboratory for PCR of the bacterial 16S RNA gene sequencing. Minimum inhibitory concentrations (MICs) to penicillin, amoxycillin and clavulanic, clyndamycin, erythromycin and linezolid, were determined by E-test on Mueller Hinton sheep blood agar in anaerobic conditions.
Results: A 32 year-old woman presented inflammation in the right breast. An initial diagnosis of a breast abscess was made and needle aspiration was attempted. Four months later, she developed a new abscess in the same place. The abscess was drained and the pus was sent for bacteriological culture. The sample was processed by standard protocols, but bacteriological investigations were negative. The patient was treated with ceftidoren. After two months, a breast magnetic resonance imaging was performed to control the evolution and the abscess was observed one more time. It was required other drainage procedure, the sample was immediately sent to the Microbiological Department. After 48 h anaerobic incubation, colonies <1 mm in diameter, grey, convex and weakly-haemolytic were obtained. The Api Rapid ID32 identified the microorganism as *Actinomyces meyeri* with the code 0521473705. The result of the definite confirmation by PCR was *A. schaalii*. According to the Clinical and Laboratory Standards Institute criteria for *Streptococcus* spp., the isolate was susceptible to all antibiotics tested.
Conclusion: This is the first case of *A. schaalii* isolate found in a breast abscess. These organisms could be missed in routine cultures due to the anaerobic and slow growth. It is recommended that the identification of *A. schaalii* be done by performing Rapid ID32A test system and 16S rRNA gene sequencing, at least until the manufacters' databases have been updated.

R2215 Clinical and epidemiological aspects of invasive *Streptococcus pyogenes* during one year

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Objective: The aim of this study was to improve understanding of the epidemiology of invasive group A streptococcal (GAS) disease.
Material and Methods: We realised a retrospective study of GAS isolates recovered from hospitalised patients with invasive disease. The isolates were sent to Carlos III Institute to typing procedures.
Results: There were 21 patients: 11 males (52.4%). The median and the mean age of patients were 40 years (range 0 to 77) and 38.47 years, respectively, with no important differences between male (40.27 mean) and females (36.5). The incidence was increased with low age range: <20 y. (33.3%), 20–50 y. (33.3%), 50–70 (9.6%) and >70 (23.8%).
The majority of isolates were recovered from blood (52.4%); skin, soft tissue infections (33.3%) and arthritis joint (4.8%) and other normal sterile sites. Skin and soft infections accounted for 52.4% of the cases (n = 11), arthritis (4.8%) (n = 1) and primary bacteraemia with no focal symptoms (28, 6%) (n = 6). 16 patients have at least one risk factor of which neoplasia accounted in n = 5, diabetes n = 3, previous traumatism n = 3, and previous surgery in n = 1. The mean case fatality rate was only a case 4.8% a woman 73 years old. Were admitted to intensive care 2 (9.5%) patients and underwent surgical intervention 3 (14.3%) patients as a part of the treatment.
Type distribution of GAS A broad range of emm types was recorded with the most common types being types, 1 (26.3%), 22 (10.5%), 3(10.5%), and 89 (10.5%). All emm 1 isolates (5) were T1, the emm 3 (2) were T3 type the emm 89 (2) T B3262 and the emm 6 (1) were T 6.
Skin and soft tissue infections were caused by emm 1, and emm 89 (20%) and 11, 22, 3, 5, 50, 6 (10%). Septicaemia isolates evenly distributed emm 1, 3, 4, 6, 77 and 87 (10%).
The cluster genes A/B/F/G/J/Z and B/C/F/G were detected in 21.1% and 15.8% of the isolates. Skin and soft infections were associated in 30% with the cluster B/C/F/G. Among the 21 strains 71.4%, (5) were speA positive and speC negative and 71.4% (10) were speC positive and speA negative. Positive speA and speC were 28.6% (2).
The rate of erythromycin-clindamycin resistance was 5%. This strain was emm 11, T11 and the B/C/F/G/H cluster gene.
Conclusions: The incidence of GAS was in the group of age <50 years and a smaller peak >70 years. Skin and soft tissue infections accounted 52.4%. A low level of macrolide resistance has been detected in this group. emm type 1 and 89 accounted for the highest percentage of invasive disease.

R2216 Changes in the activated T-lymphocytes in patients with Mediterranean spotted fever and Lyme borreliosis aged 60+ years

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Introduction: Multiple clinical observations lead to the conclusion that old age is the most widely distributed type of immunodeficiency. Immunocompetence decreases with the ageing, i.e. the immune system starts to lose some of its functions in the process of growth and becomes unable for fast and effective response to stimulation. Age dependent changes in the immune system are observed at all levels – chemical changes in the cells, differences in the types of the surface cell proteins, changes in the organs.
Objectives: To specify the changes in the activated T-lymphocytes in patients with Mediterranean spotted fever (MSF) and Lyme borreliosis in the age 60+.
Methods: We investigated the cell immunity of 30 patients of age over 60 years using immunofluocytometry – 20 of them with MSF and 10 with Lyme borreliosis. The control group consisted of 10 young patients and 10 healthy individuals over the age of sixty.
Results: Increased number of the activated cytotoxic T-lymphocytes (CD3+/HLA-DR+) was found in the patients over 60 years of age with MSF and also in patients under 60 years with MSF. In the elderly patients with Lyme borreliosis and in the healthy 60+ controls activated T-lymphocytes are two times less. There is statistically reliable difference between activated T-lymphocytes in patients with Mediterranean spotted fever (MSF) over sixty and the control group of healthy elderly individuals, $p < 0.01$. Straight correlative dependence exists between activated T-lymphocytes (CD3+/HLA-DR+) and age in healthy individuals and patients of 60+ with MSF. In patients over 60 years with Lyme borreliosis and in patients under 60 years with MSF this dependence is reverse. There is a moderate straight dependence between the activated T-lymphocytes and the severity of the disease in patients under 60 years of age with MSF.
Conclusions:

- Activated T-lymphocytes increase their number in all patients with Mediterranean spotted fever. This is related with the increased activity against the infected endothelial cells and macrophages. Those activity probably is crucial for the healing process.
- The observed differences between the activated T-lymphocytes in elderly patients with Mediterranean spotted fever and elderly healthy individuals are statistically reliable.

R2217 Prospective evaluation of rickettsioses in the Trakya region of Turkey, 2008

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Objectives: Prospective surveillance, serologic and molecular diagnosis of rickettsioses in the Trakya Region of Turkey has been conducted by Trakya University and Université de la Mediterranée, Marseille, France since 2003. For three years, we have started to use molecular methods for the diagnosis of spotted fever group(SFG) rickettsioses in the Trakya University Hospital, a tertiary-care hospital in Edirne, Turkey.
Materials and Methods: From May to September 2008, 28 patients with Mediterranean spotted fever (MSF) were admitted to the Trakya University Hospital. SFG rickettsioses were diagnosed clinically. Before treatment, punch biopsy from skin lesions (eschar or maculopapular rash) was performed. Serum specimens were tested by microimmunofluorescence assay MIF) using a commercially available antigen (*R. conorii* IFA IgG; Focus Technologies, USA). DNA was extracted from skin biopsies using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany). Standard PCR was performed with primers suitable for hybridisation within the conserved region of genes coding for outer membrane protein A (ompA) and citrate synthase (gltA).
Results: The average age of 28 patients (12 male and 16 female) was 57±17. All the patients had high fever and maculopapular rash (including the palms or soles of the feet). Twenty three patients (82%) had eschar. Twenty patients (71.4%) had clinical scores ⩾25. One patient presented with meningoencephalitis. Twenty one patients (75%)had significant antibody titers against Spotted Fever Group Rickettsiae. Twenty three patients (82%)accepted skin biopsy. PCR experiments were positive in 18(78%) out of 23 biopsy samples.
A 28 years old man with possible tick contact presented with high fever, headache, nausea and a few macular rash on his arms without eschar; leukopenia, thrombocytopenia, elevated ALT, AST, LDH, CPK levels, low level of CRP were determined. RT-PCR test for Crimean-Congo haemorrhagic fever virus RNA was negative but antibody titers against Spotted Fever Group Rickettsiae increased more than four folds.
Conclusion: Trakya Region is an endemic area for rickettsioses with a population of one million. Since 2001, 124 patients were diagnosed as SFG rickettsioses. Differential diagnosis of tick-born diseases is important in Turkey, as Crimean-Congo haemorrhagic fever is also reported since 2003.
This study was supported by TÜBİTAK SBAG-K-78 (104S290).

R2219 Risk of Crimean-Congo haemorrhagic fever among healthcare workers

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Objectives: Crimean-Congo haemorrhagic fever (CCHF) is a potentially fatal disease. The disease caused by a virus belonging to Bunyaviridae family. CCHF has been reported from more than 30 countries in Africa, Asia, south-eastern Europe, and the Middle East. The disease has been seen since 2002 in middle and east of Turkey. The number of patients is getting increase by years. The main transmission routes of the virus are tick-bite and contact with tissues, body fluids and blood of infected animals. Contact with a patient's blood or excretion is another route of transmission. The aim of this study was to investigate seroprevalence of CCHF in health care worker (HCW) of three hospitals which in CCHF patients were hospitalised and followed up.
Methods: This study was executed in Ankara Training and Research Hospital, Kastamonu State Hospital and Corum State Hospital. The sera of the HCWs' who are working in infectious diseases and clinical microbiology, emergency and laboratory departments of these hospitals were tested. A questionnaire was fulfilled for each HCW. Presence of CCHF IgG was investigated in the sera samples by ELISA (Vectocrimean-CHF-IgG, Bectop, Russia).
Results: Totally 90 HCWs were included in the study. Thirty-eight (43.2%), 31 (35.2%) and 21 (23.9%) HCWs were from Corum State Hospital, Kastamonu State Hospital and Ankara Training and Research Hospital, respectively. Distribution of the profession of the HCW's was as follow: 41(46.5%), nurses 18 (20.5%) physicians, 11 (12.5%) laboratory technician and 18 (20.5%) other. Forty-seven (52%) HCWs were from Infectious and Clinical Microbiology Department, 34 (38%) were from emergency department, 9 (10%) were from laboratory. Needle-stick injury was detected in two HCWs, both of them had received ribavirin prophylaxis. They were negative for CCHF IgG. One sera sample was found as positive for CCHF IgG. This HCW was working in blood-draw department and he had a history of contact with blood and needle-stick injury. He had no symptoms or clinical findings of CCHF.
Conclusion: In the literature, CCHF outbreaks by nosocomial transmission have been reported. In Turkey, there have been eight reported cases between 2002 and June 2008. One of them was died. We found a low rate of seropositivity in HCWs (1%) in endemic regions. This might be related to compliance with application of standard control measures.

R2220 Evidence for tick-borne encephalitis virus infections in Bulgaria

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Objectives: Officially, there are no reported cases of tick-borne encephalitis virus (TBEV) infections in Bulgaria. Nevertheless, clinically

compatible disorders have been described but have not been laboratory confirmed.
Materials and Methods: Two hundred and seven serum samples from 120 patients with febrile syndromes were evaluated for IgM and IgG antibodies against TBEV by a commercial enzyme-linked immunosorbent assay (ELISA) kit.
Results: Seroconversion of TBEV IgM antibodies was detected in 20 (16.7%) of 120 patients and seroconversion of both classes, IgM and IgG, antibodies was confirmed in other 22 (18.3%) of the patients. TBEV IgG antibodies with no dynamics in the paired serum samples were found in 26 (21.6%) of the patients. Clinical manifestations in seroreactive patients were compatible mostly with viral meningitis and Mediterranean spotted fever.
Conclusions: Our results suggest the presence and wide distribution of human TBEV infections in Bulgaria.

Infection control

R2221 Information technology and infection control: a project for an active computer-assisted microbiology surveillance

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Objectives: The correct management of data represents one of the most relevant factors in the processes of risk-management and control of hospital infections(HAI). Informatised laboratories, a privileged observatory for HAI surveillance, have contributed to optimise the processes of data collection and elaboration. A project aiming at transforming passive laboratory surveillance into an active "virtual" process started in the hospitals of Trentino.
Methods: A dedicated software was developed which, once integrated with the databases of the laboratories, can carry out a systematic and real-time research of the possible epidemic events, of the warning/alert events and integrated clinical data. At the same time, it can periodically control antibiotic-resistance and infection prevalence. This software is made up of three components: 1)Import, which imports data in real time through a connection to the data-base of the laboratory; 2)Elaboration, which filters the alarms on the basis of the established rules; 3)Report, which produces the report according to the queries defined by the user. These data are sent to the ward in electronic format using the visualisation system of the laboratory reports and, at the same time, a notification file is produced for the infection control unit. In the second phase of the process, the wards give the notification with the clinical information back to the laboratory and it is filed in a normalised and standardised data structure for a real-time monitoring which also constitutes a historical database for longer periods.
Results: In the first three months the surveillance system has highlighted its considerable potentialities. 4 outbreaks have been precociously detected, as well as numerous microorganisms and alert events followed by the immediate activation of processes and precautions aiming at circumscribing and limiting their diffusion. A major sensitivity of health-workers to HAI and bacterial resistance problems and a better integration between laboratories, wards and infection control personnel were highlighted as well.
Conclusions: The described system has proved able to increase the monitoring, control, treatment and prevention power against hospital infections. Such system integrates the surveillance programs which are already active with a tool able to detect outbreaks precociously and to provide a rapid alert system for the ward, which can thus precociously activate the necessary precautions to limit infectious events.

R2222 Value of procalcitonin, C-reactive protein and leukocyte cell count for the diagnosis and antibiotic management of intensive care unit-acquired infections

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Objectives: The patients in Intensive Care Units (ICU) are subjected to various inflammatory factors, a fact that makes the diagnosis of infection difficult. The aim of this prospective study was to compare the value of Procalcitonin (PCT) to that of C-Reactive Protein (CRP) and White Cell Count (WBC) for the diagnosis of ICU-acquired infections.
Patients and Methods: 104 patients who stayed for more than 72 hours in the ICU and had no infection at admission were included in the study. Serum CRP and PCT measurement and WBC counts were performed in parallel every three days. Blood cultures were taken as clinically indicated.
Results:
- Normal CRP levels and WBC counts were accompanied by normal PCT levels.
- Out of 63 positive blood cultures, PCT was normal in only 7 cases, in each of which the CRP level was <5 mg/dl and WBC counts were normal. It was considered that these cultures were probably contaminated.
- The infection-free patients had normal or slightly raised average PCT values but significantly elevated CRP levels and WBC count throughout their stay in the ICU.
- The infected patients had significantly elevated PCT values during the infection, which decreased quickly after effective treatment and was completely normalised on discharge from the ICU. Among these patients' population, average CRP levels were in the pathological range even on discharge from the ICU.

Conclusion: PCT measurement is a more specific marker in the diagnosis of infection in ICU than CRP and WBC count, allowing better selection of patients who should receive antibiotic therapy.

R2223 Nosocomial pneumonia on general medical ward in a tertiary-care hospital

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Objective: To describe the demographic, clinical, and microbiologic characteristics of patients who develop nosocomial pneumonia on general medical ward of a tertiary-care hospital.
Methods: We study retrospectively, the files of our patients hospitalised in our Department for nosocomial pneumonia, using a standard case definition.
Results: During these years (1986–2006) we identify 1056 nosocomial pneumonias in our patients. The mean age of these patients was 63±12 year (53% were males). Bacteraemia was identified in 467 patients (44%) of 612 episodes, and 238 (51%) grew potential pathogens from respiratory specimens. Eighty eight patients (37%) required transfer to the intensive-care unit (ICU), and 20 (22%) received mechanical ventilation. By multivariate analysis, patients with *Staphylococcus aureus* isolated from respiratory secretions were more likely to require ICU admission. The overall mortality rate was 20% (19/88), with a directly associated mortality of 14% (17/88). Patients who died were older, more frequently resided longer on a medical ward, and had a greater mean number of co-morbidities. These patients often were treated no aggressively and were not considered candidates for ICU admission due to advanced age and poor underlying clinical status.
Conclusion: Although the morbidity of nosocomial pneumonia in this population was high, as evidenced by high rates of transfer to ICU, the directly associated mortality was relatively low. Admission to ICU requires further study for those patients to identify preventive measures that could decrease the morbidity in this group. Interventions to prevent pneumonia or to improve prognosis may not be feasible for the majority of these old patients who die from nosocomial pneumonia

R2224 Impact of economic modelling and infection prevention protocol compliance on blood culture contamination rates

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Objective: Contaminated blood cultures result in delay in optimal clinical management and associated healthcare costs. An economic modelling was undertaken to gather an impression of associated wastage of resources and coupled to a 3-year surveillance of all blood cultures processed in the microbiology laboratory.
Methods: Data on all blood cultures processed over 3-years [1.10.05–31.10.08] was collected from laboratory database. A working definition of contaminants was used to compare all positive blood cultures. Rates of blood culture collectionstions, trends in key pathogens, contamination rates and effect of infection prevention interventions were analysed.
Results: A total of 25325 blood cultures were processed over the 37 month period. Of these, 5507 (21.75%) were positive for significant bacterial growth. Contaminants were identified 2896 blood cultures (52.59% of positive blood cultures, 11.4% of all blood cultures taken). The monthly rates of contamination are displayed in the graph below, along with the linear regression. The results show a significant downward trend in the results from the 3 year period ($r = -0.42$). However we were unable to demonstrate a further increase in the downward trend following introduction of the aseptic policy. Rates of MRSA appeared to have decreased in the study period. Rates of blood cultures positive for MRSA (as a percentage of total positive cultures) has decreased from an average of 3.58% in 05–06, 3.15% in 06–07, to 2.14% in 07–08 ($r = -0.44$). Rates of pseudomonal bacteraemia appear to have increased in the study period when plotted graphically, however no statistically significant increase was identified. Rates of candidal septicaemia do not show a significant increase or decrease (mean 2.41% of all positive blood cultures).
Discussion: The overall downward trend of blood culture contaminants is encouraging, however this conclusion must be treated with caution. We were unable to demonstrate a significant improvement in rates following the introduction of the new policy; however the study may have been undertaken too soon after the policy change to detect any affect.

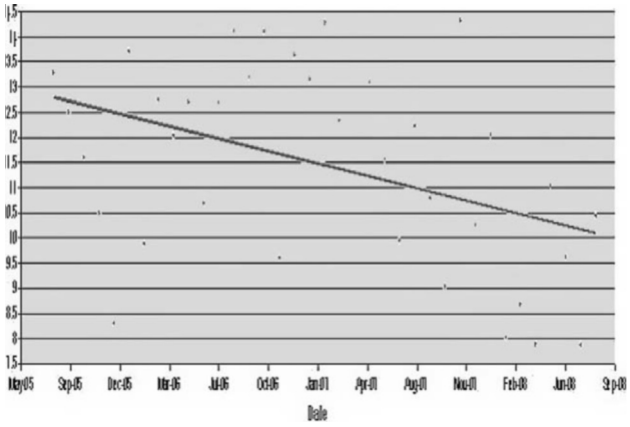

Figure: Monthly contamination rates.

R2225 Control charts with days between episodes in surveillance of nosocomial bacteraemia in intensive care units: a way to intervene

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Background: Nosocomial infections are often seen after invasive procedures in hospitals; especially in intensive care units (ICU) were patients frequently are given intravenous lines, catheters, drainages, etc., besides having surgery performed recently. Surveying nosocomial bacteraemia draws attention to the problem, and makes it possible to study the effect of various interventions. Creating control-charts for nosocomial bacteraemia in intensive care units enables the staff to follow the rate of infection (almost in real-time) and intervene if an increased incidence is observed.
Materials and Methods: Bacteraemia is defined as a clinical episode with one or more positive blood cultures, given significance by a clinical microbiologist and the attending physicians, and nosocomial bacteraemia is defined as bacteraemia from an the infection which was not present or incubating at admission to the hospital. As part of a collaborative network between three departments of clinical microbiology, a semi national database of bacteraemia with numerous clinical and paraclinical information has been established, making this special surveillance possible. In this study, data from 2006–2008 (2008; three quarters) is presented for two ICU's. In the autumn of 2007, the antimicrobial treatment was changed for patients undergoing abdominal-surgery.
Results: The mean incidence (bacteraemic episodes/1000 bed days) for the two departments, Bbh-ICU and Hvh-ICU, were: Bbh-ICU: 10.8 and 13.6, and Hvh-ICU: 5.8 and 9.1, in 2006 and 2007, respectively.
The mean number of days between episodes was: Bbh-ICU: 20.3, 18.3, 27.4 and Hvh-ICU: 30.4, 24.3, 27.4, in 2006, 2007, 2008 (2008; three quarters), respectively.
Using the programme CHARTrunner® (PQ Systems, Miamisburg, OH, USA) data was analysed statistically. It was possible to recognize both statistically significant clusters of nosocomial episodes in the ICU's, but also a significantly decrease in nosocomial infections ($p < 0.01$) after change in recommendations for antimicrobial use in abdominal surgery.
Conclusion: Control charts showing days between episodes are effectively used for surveillance of nosocomial bacteraemia in intensive care units. Deviations in occurrence can be identified as clusters of episodes and thereby makes it possible to quickly intervene.

R2226 Effect of shorter antimicrobial prophylaxis+using intestinal cleaning+using single use sterile gels on infections developing after transrectal prostate biopsies

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Background: The aim of this study was to evaluate the effects of shotrter antimicrobial prophylaxis+using intestinal cleaning+using single use sterile gels on infections developing after transrectal prostate biopsies in a tertiary-care educational hospital.
Methods: After observing an increase in infections developing after transrectal prostate biopsies at the end of 2007, biopsies were stopped and procedures before and during the biopsies were evaluated. Infections developed in the last six months were retrospectively evaluated by using records of mictobiology, radioogy and urology. Sterilisation & disinfection, antimicrobial prophylaxis regimens, intestinal decontamination procedures and routine biopsy procedures were reevaluated in cooperation with with the corresponding clinics. Afterwards we implemented three main changes: (i) Five day lasting antimicrobial prophylaxis was changed to one day lasting prophylaxis (ciprofloxacin 500 mgx2+ornidazole: 2×1 gr starting two days before the biopsy and lasting until the third day of it + amikacin 500 mg !×1 during the biopsy, to the ciprofloxacin 500 mg×2 + ornidazole given 2 h before the biopsy.) (ii) we started to implement intestinal cleaning one day before the intervention by using enema [Fleet enema adult lavman, Kozmed, Turkey, implemented twice on the day (8 h apart) before the biopsy]. (iii) we started to consume single use sterile gels during biopsy (instead of multiple use gels). Patients were diagnosed as clinical or microbiologically confirmed infection according to CDC criteria.
Results: Infection rate during the preintervention six months period was 24/294 (8.1%, 17/294 microbiologically confirmed infection, 7/294 clinically diagnosed infection)and it was 7/186 (3.7%, 5/186 microbiologically confirmed infection, 2/186 clinically diagnosed infection) in the postintervention four months period (p: 0.056 with Chi-square test).
Conclusion: When we compared the two periods, there was a prominent (more than 50% but $p > 0.05$) decrease in the post-biopsy infection rates.

Our findings suggest that shorter antimicrobial prophylaxis regimens, sterile gel consumption and intestinal cleaning may be useful in the control of infections developing after transrectal prostate biopsies.

R2227 The hand hygiene compliance of healthcare workers and detection of MRSA hand colonisation by using CHROMagar MRSA

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Aim: In this study, we aimed to investigate the hand hygiene compliance of the health care personnel (HCPs) during their routine patient care, determine the MRSA hand colonisation of the HCPs, investigate the effect of different hand hygiene products on MRSA colonisation, and to compare the chromogenic agar results with conventional methods.
Methods: The study was held with health care workers (HCWs), who usually work in the departments like ICUs, infectious diseases department and surgical departments. HCWs were investigated during their routine patient care and hand cultures were taken before and after hand wash/hygiene. Soap, alcohol based and chlorheksidin based hand antiseptics were used for hand hygiene. Fingertip and inoculation to plates with swab techniques were used for each individual when getting the cultures. Both media were containing CHROMagar MRSA. Suspected MRSA colonies in these media were confirmed with convantional methods.
Results: Thirty nine (39%) male and 61 (61%) female, totally 100 HCWs were included in the study. Mean age was 32.7±5.2. Of the study group 33% was physicians, 38% was nurses and 29% was other HCWs. Fifteen percent of them used alcohol based hand rubs while 35% used clorheksidin and 50% preferred soap and water for hand wash. Clorheksidin was the main choice in operation rooms while soap was for other departments. Among HCWs there was no difference regarding antiseptic preferences. Mean hand hygiene duration time was 44.6±29.9 seconds. Sex, occupation and working durations were not statistically significant for hand hygiene duration. However, in the intensive care unit hand washing time was significantly lesser than the other departments ($p < 0.5$). Of all staff, MRSA was detected in 39% and 13% with fingertip method and in 11% and 6% with plate media, before and after hand hygiene respectively. Log reductions of the MRSA colonisation with alcohol based hand rub, soap and clorheksidin were an average of 4.1 log10, 2.2 log10 and 2.7 log10 respectively. Effect of the duration of the hand hygiene on log reduction of the MRSA was not found to be statistically significant.
Conclusion: We found a high ratio (39%) of hand colonisation with MRSA among our hospital staff. It is also showed that the colonisation can be reduced up to 66% with hand hygiene. The fingertip technique is found to be superior to inoculation to plate media. The study is also among the first studies which use chromojenic media for hand cultures.

Clinical epidemiology of nosocomial infections (POWI, VAP, UTI, BSI, ...)

R2228 Neurosurgery-related bacterial meningitis – a 10-year review

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Objectives: Bacterial meningitis is an uncommon complication of neurosurgical procedures associated with relevant morbidity and mortality. Thus epidemiology, aetiology and prognosis of this infection were studied.
Material and Method: we retrospectively studied 148 patients treated for neurosurgery related bacterial meningitis (NSBM) in the Infectious Diseases Hospital from Iasi, Romania, between 1998 and 2007.
Results: 16.9% of all bacterial meningitis treated in our hospital during the period of the study were NSBM. An increase of the annual number of NSBM and a decrease of community acquired bacterial meningitis was also noted. Males were more frequently involved (62.1%); 41.4% of patients were children (12.1% under one year old). The aetiology of the NSBM could be established in only 49% of the patients; staphylococci were involved in 16.2% of cases ((*S. aureus* – 11.7%, coagulase-negative staphylococci – 4.5%). Most of the staphylococcal isolates were MRSA (55.6%). Gram negative bacilli were involved in 6.2% of cases. The mortality rate of NSBM was 8.43% and did not decrease during the study period.
Conclusion: the annual number of neurosurgery related bacterial meningitis is increasing, the most common pathogens were staphylococci and the mortality rate was lower than in community acquired bacterial meningitis

R2229 The evaluation of catheter-related bloodstream infections in neurology and neurosurgery intensive care units

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Objectives: Catheter related bloodstream infections (CR-BSIs) are considered the most common serious complication associated with the use of central venous catheters (CVCs). We aimed in this study to determine the rate, epidemiological and microbiological characteristics, risk factors and outcomes of the CR-BSIs in patients hospitalised in Neurology and Neurosurgery Intensive Care Units (ICUs).
Methods: This prospective study was carried out between January 2007 and January 2008. All the patients hospitalised in Neurology and Neurosurgery ICUs with newly inserted central venous catheter were enrolled into the study. CR-BSI rate, risk factors for CR-BSIs and for mortality were studied.
Results: During the study period, 199 CVCs in 148 patients were followed. Eighty-two patients were female (55.4%), mean age was 58.7±21.8 years. Sixty-seven patients (45.3%) hospitalised in Neurology ICU and 81 (54.7%) in Neurosurgery ICU. Mean hospital stay in ICU was 15.6±15.3 days. Eighty-nine (60.1%) patients were died. Mean duration of catheterisation was 8.5±5.2 days. There were 32 episodes of CR-BSI in 29 patients. Total catheter days were 1703 days. CR-BSI rate was 20.3 per 1000 catheter days. In univariate analyses comparing patients with CR-BSI and without CR-BSI, we found that prior antibiotic therapy rate was higher ($p = 0.02$) and mean hospital stay in ICU was longer ($p < 0.001$) in patients with CR-BSI. In patients hospitalised in Neurology ICU, CR-BSI risk was 2.4 times higher than Neurosurgery ICU patients ($p = 0.004$). Catheters which kept in place for more than 7 days were increased the risk of CR-BSI 6.3 times ($p < 0.001$). In multivariate analyses, catheterisation day and hospital stay in ICU were found independent risk factors ($p < 0.001$). The mortality rate was higher in patients with CR-BSI (79.3%) than the patients without CR-BSI (55.5%) ($p = 0.019$). In univariate analyses comparing fatal patients with CR-BSI and non-fatal patients with CR-BSI for mortality risk factors, we found that mortality rate was higher in patients aged greater than 60 ($p = 0.017$), in patients hospitalised in Neurology ICU ($p = 0.006$) and in patients receiving TPN ($p = 0.03$). In multivariate analyses, being aged greater than 60 was found independant risk factor for mortality ($p = 0.037$).
Conclusion: Catheterisation day and hospital stay were found independent risk factors for catheter-associated blood-stream infections, and age greater than 60 years was found independent risk factor for mortality.

R2230 Surveillance of vascular catheter and total parenteral nutrition-related infections

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Objectives: Two vital components of critical care are the use of central venous catheters (CVC) and total parenteral nutrition (TPN). In the intensive care unit and surgical ward the patients often need for survival and recovery. The use of CVC for the administration of TPN is a

risk factor of catheter-related infections (CRIs) that are associated with increased morbidity and mortality, prolonged hospitalisation, and increased medical costs. We analysed the results of an active surveillance system of nosocomial infections associated with CVC for the administration of TPN in patients hospitalised in intensive care units (ICU) and surgical wards (SW) of a country hospital (720 beds). We evaluated the incidence rate of CVC/TPN related infections and the microorganisms involved.

Methods: The surveillance was based on CDC's Guideline of Intravascular Catheter-related Infections. The study comprised patients hospitalised between January 2006 and March 2008 in two surgical wards (SW1, SW2) and in an intensive care unit (ICU). All CVCs associated to TPN were prospectively followed until CVC removal. Standard clinical and microbiological criteria were used to define colonisation and infection.

Results: 348 patients receiving TPN, comprising 6559 central venous catheter-days, were observed. The infections recorded were 37and the incidence rate/1000 catheter-days was 5.64‰. The data of each unit were analysed until March 2007. In this period in the ICU 5 cases of CVC/TPN related infections were recorded; they correspond to a lower incidence rate/1000 catheter-days (4.57‰) in comparison to the surgical wards, where the highest incidence rate/1000 catheter-days was recorded, especially in the wards with a high activity of abdominal surgery (12.71‰ in SW1 and 7.76‰ in SW2). The average catheterisation was higher in the patients with infections than in the patients without infections (57.88 vs. 56.78) but not really significant. The microorganisms more frequently isolated in microbiological cultures was *Staphylococcus* coagulase-negative, but also *Candida* spp., *S. aureus*, *S. maltophilia* and *Enterobacter* spp. were detected.

Conclusions: The results show that the highest frequency of CVC/TPN related infections was in the surgical ward of gastrointestinal surgery, confirming that gastrointestinal dysfunction and abdominal surgery are associated with a high risk of CRIs, but this can also underline a need to improve the infection control procedures in the ward.

R2231 Device-associated nosocomial infection rates in three different intensive care units in a training and research hospital, Ankara

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Objectives: Patients admitted to intensive care units (ICUs) have a high risk of infection associated with their underlying conditions and the invasive medical procedures that they undergo. In this study device associated infection (DAI) rates of three different ICUs in our hospital were calculated and compared with both Turkish National Nosocomial Infection Surveillance Reports (TNNISR) and National Healthcare Safety Network (NHSN) Report.

Methods: The study was conducted in our hospital, from August 2007 to December 2008. A prospective surveillance of DAIs in Neurology ICU (NR-ICU), Neurosurgery ICU (NRS-ICU) and a Medical/surgical ICU (MS-ICU) was conducted. Nosocomial infections were identified according to CDC criteria. DAI rates were calculated as the number of infections per 100 ICU patients and per 1,000 device-days.

Results: Data on 1841 patients who were hospitalised in NR-ICU, NRS-ICU and MS-ICU with a total of 11959 patient-days were analyzed. The mean overall infection rate per 100 patients was 29.1 infections, and the mean infection rate per 1,000 patient-days was 44.7 infections. Devices utilisation ratios and the rates of ventilator-associated pneumonia (VAP), catheter-associated urinary tract infection (CA-UTI), and catheter-associated bloodstream infections (CA-BSI) of three different ICU were shown in Table 1.

The most common cause of VAP in all three ICUs was *Acinetobacter baumannii*, whereas *Candida* spp. was mostly isolated microorganism that caused urinary tract infections in those ICUs. *A. baumannii*, *Candida* spp. and *Enterococcus* spp. were the most frequently isolated organisms from CA-BSI from the patients hospitalised in MS-ICU, NRS-ICU and NR-ICU, respectively.

When compared with TNNISR device associated infection rates were as follows: CA-UTI rates of all the ICUs were between 75%-90%. CA-BSI rates detected from MS-ICU and NRS-ICU were between 25%-50%, whereas the same rate detected from NR-ICU was between 75%-90%. VAP rates were as between 25%-50%, 75%, and 75%-90% for NRS-ICU, MS-ICU and NR-ICU, respectively. We found DAI rates in our ICUs higher than those reported by the NHSN system.

Conclusion: These results suggest that, DAI rates in our three ICUs were higher than NHSN data and equal/higher than TNNISR data. These data alerted us that we must improve education program for all healthcare workers, rationalise device utilisation, and establish more-effective infection control practices and policies in our ICUs.

Table 1. DAI and device utilisation ratio of ICUs

ICU	Ventilator utilisation ratio	VAP rate	Urinary catheter utilisation ratio	CA-UTI rate	SVK utilisation ratio	CA-BSI rate
Medical/surgical	0.49	32.94	0.88	14.32	0.62	11.56
Neurosurgery	0.18	21.74	0.90	13.80	0.63	5.29
Neurology	0.15	53.16	0.98	21.00	0.31	19.27

VAP: ventilator-associated pneumonia; CA-UTI: catheter-associated urinary tract infection; CA-BSI: catheter-associated bloodstream infection.

R2232 Vertebral osteomyelitis post-vertebral surgery. A descriptive and comparative study of 100 cases

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Objectives: To study the epidemiology, clinical and diagnostic features, treatment and outcome in patients with postvertebral surgery vertebral osteomyelitis (PSVO), and to compare with other pyogenic vertebral osteomyelitis (PVO).

Methods: Study of 514 patients diagnosed of vertebral osteomyelitis between January 1983 and November 2008. Inclusion criteria: 1) spinal inflammatory pain or fever and spinal pain on physical examination, 2) imaging findings compatible with VO, 3) aetiologic diagnosis. We analyse epidemiology factors, clinical features, radiologic and analytic parameters, microbiology isolates, treatment and outcome. Patients were followed since 12 months. Statistical analysis: chi-square and Fisher's tests.

Results: PVO was diagnosed in 267 patients (52%); and of them 100 patients (37%) were PSVO. Underlying disease: 1) diabetes mellitus: 10 patients PSVO (10%) vs 44 PVO (26%)($p = 0.001$); 2) immunosupresion: 7 patients PSVO vs 43 PVO ($p < 0.001$). Previous bacteraemia: 3 patients PSVO vs 53 (32%) with PVO ($p < 0.001$). Duration symptoms: median 22 days (35 d. POV, $p = 0.004$). Clinical features: Inflammatory back pain 88 PSVO vs 157 (95%) PVO ($p =$ ns), fever 54 PSVO vs 120 (72%) PVO ($p = 0.002$), neurological symptoms and signs 78 patients (78%) PSVO vs 88 (53%) PVO ($p = 0.001$). Blood culture positive: 21/46 (46%) PSVO and 79/129 (61%) PVO ($p =$ ns). Bone biopsy culture positive: 37/41 (90%) PSVO and 71/91 of PVO (78%) ($p =$ ns). Culture of other samples: 74/75 (99%) PSVO vs 59/68 (87%) of PVO ($p =$ ns). Aetiological agents: Gram-positive cocci 75 (64%) (coagulase negative *Staphylococcus* spp. 36, *S. aureus* 31, other Gram-positive cocci 8), Gram-negative bacilli 23 (20%)(*Pseudomonas aeruginosa* 7, *Escherichia coli* 6, *Proteus mirabilis* 5, others 5), anaerobic bacteria 18 (15%)(*Propionibacterium* spp. 7, *Bacteroides fragilis* 3, others 8), and other 1. Polymicrobial isolates: 17 cases. For 64 patients with PSVO surgical treatment was needed vs 74 (44%) with PVO ($p = 0.003$). Severe functional sequelae: 41 patients with PSVO vs 59 (35%) ($p =$ ns). Relapse: 1 patient. No deaths.

Conclusions: 1) PSVO is an important segment of the PVO. 2) Underlying diseases and previous bacteraemia are less frequent than others PVO. 3) The duration symptoms is smaller, and the neurological symptoms and signs are more frequent than PVO. 4) PSVO is caused by a variety of organisms. 5) Diagnostic yield of microbiological techniques

is very high. 5) Patients with PSVO need of surgical treatment more frequent.

R2233 High prevalence of colonisation of vancomycin-resistant enterococci in adult haematology unit patients

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Objective: Vancomycin-resistant enterococci (VRE) are widespread worldwide. Colonisation and infection with VRE are problems in hospitals worldwide. Despite growing concern about VRE as nosocomial pathogens, they are rarely isolated in Polish hospitals. To determine the prevalence of VRE colonisation in adult haematology unit patients during a 5-year period at the Medical University Hospital, Gdansk, Poland.
Methods: Anal swab culture were collected from patients in adult haematology unit between October 2003 and October 2008. Samples were collected from patients whit more than 2 days of admission in these units, and repeated every 6 days until discharge. Samples were cultured on a bile esculine agar with vancomycin (6 mg/L) for VRE. Strains were then reincubated in a blood agar medium and identified by Vitek (bioMerieux). Minimal inhibition concentration against vancomycin, teicoplanin and linezolide was determined by an E-test.
Results: In adult haematology unit there were 5896 samples from 593 patients. VRE were isolated from 234 (37%) patients. Among total 234 non-repetitive VRE isolates, 223 were identified as *E. faecium*, 6 as *E. casseliflavus*, 4 as *E. gallinarum* and 3 as *E. faecalis*. All isolates *E. faecium* expressed a VanA phenotype. Only 3 isolates were resistante to linezolide.
Conclusion: Vancomycin-resistant enterococci are a major concern in our adult haematology unit. VRE colonisation must be monitored and risk factors should be determined. They are useful for screening hospitalised patients for VRE colonisation in order to establish prevention and control measures.

R2234 Healthcare-associated infections in a medical-surgical intensive care unit, in a research and training hospital

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Objectives: Healthcare associated infection is a common problem in intensive care medicine. Some factors such as severity of underlying disease, altered consciousness, impaired protective reflexes, concomitant immunsupression and exposure to invasive devices increases the risk of healthcare associated infections.
In this study, we aimed to assess data on the epidemiology of healthcare associated infections (HAI) among patients in a medical/surgical intensive care unit (MS-ICU).
Methods: The study was conducted in Ankara Training and Research Hospital, between September 2007 and December 2008. The patients, treated for more than 48 hours in 10-bed MS-ICU were enrolled into the study. The patients were followed until death or three days after discharge by prospective daily surveillance. HAI were identified according to CDC criterias. Identification and antimicrobial susceptibility testing of isolated microorganisms were performed by a Vitec-II system (Biomerio France).
Results: During the study period, 682 patients were hospitalised in MS-ICU with a total 3446 patients days. Among these patients, 127 HAI were detected. HAI rate was 18.6% and hospital infection incidence density was 36.9 per 1000 patient's days.
Ventilation associated pneumonia (40.9%), urinary catheter related urinary tract infection (31.5%), catheter related bloodstream infection (17.3%), pneumonia (7.9%) and surgical site infection (2.4%) were the frequently detected infections. Ventilator associated pneumonia, urinary catheter associated urinary tract infection and central line associated bloodstream infection rates were 31.38, 12.89, 10.39 per 1000 devices days respectively. Utilisation ratios of ventilator, urinary catheter and central lines were as follows 0.48, 0.88 and 0.61, respectively. All of these results were higher than NNIS results.
The most common isolated microorganisms were *A. baumannii* (35.2%), *E. coli* (17.2%) and *Candida* spp. (16.4%). The frequency of resistance of *Staphylococcus aureus* isolates to methicillin (MRSA) was 80%. Extended spectrum β-lactamase production was detected in 43% of Gram negative enteric bacteria isolates. Carbapenem resistance was 86% in *A. baumannii* isolates.
Conclusion: In this study, ICU-acquired infection rates were found higher when compared with NNIS results. These high rates alerted us to take immediate precautions for decreasing device utilisation and device-associated infection rates in our MS-ICU.

R2235 Neonatal sepsis at a neonatal care unit in the Black Sea region of Turkey: a two-year analysis

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Objective: The pathogens of neonatal sepsis vary with geographic area, time, and individual neonatal intensive care units. Therefore, knowledge of related factors and the pattern of bloodstream infection can help to determine the antibiotic prescribing policy and infection control procedures. This study aimed to determine the prevalence, microbial epidemiology of neonatal sepsis, and related factors in our centre.
Method: The retrospective study reviewed records of patients with culture-proven sepsis from January 2007 to December 2008 at neonatal intensive care unit (NICU) in Samsun Maternity and Children's hospital in Black Sea Region, Turkey. Blood cultures were done using BacT/ALERT 3D system. Isolated pathogens were identified and included in the study.
Results: During the study period 3173 neonates were admitted to the NICU. From 1996 taken blood cultures, 161 bacteria strains were isolated. Of these, 43 (29.8%) were evaluated as contaminated and 118 episodes culture proven septicaemia were identified. Gram-positive organisms accounted for 57.6% (68/118) of the positive culture results, Gram-negative organisms accounted for 40.7% (48/118), and yeast for 1.7% (2/118). *Pseudomonas* and *Klebsiella* spp. weren't observed at patients with neonatal sepsis.
The rate of neonatal septicaemia was 3.7% (118/3173) and the mortality rate of these 19.5% (23/118). Neonatal sepsis mortality in 73.9% was caused by Gram-negative organisms, which were found resistant to commonly used antibiotics. The risk of death from neonatal sepsis was 7.24‰ (23/3173) for admitted patients. Sixty nine percent of the cases were inborn patients. During the study period total number of live births in the hospital was 15504, resulting in a hospital-based neonatal sepsis incidence of 5.3‰ for in-born patients. The total number of live births with low birth weight (LBW) (under 2500 g) during the study period was 7.3%, however 51% of sepsis cases were LBW. The male to female ratio was 1.53.
Conclusions: Male babies and babies with LBW had greater neonatal sepsis rate, suggesting a greater care to be given to them. Since death cases were caused by Gram-negative bacteria, antibiotic that is effective for these help the mortality rate to decrease. Understanding the local epidemiology of neonatal sepsis can lead to the development of better medical practices, especially more appropriate choices for empiric antibiotic therapy, and may contribute to improvement of infection control practices.

R2236 The effect of transfusion on the rate of sepsis in patients undergoing cardiac and vascular surgery at a tertiary care centre in India

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Objectives: It has been reported that perioperative transfusion of leukocyte-containing blood components is a risk factor for the development of postoperative bacterial infections. The purpose of this study is to analyze the rate of sepsis in patients undergoing cardiac and vascular surgery and to study the correlation of blood transfusion, duration of surgery and length of stay in the ICU with the rate of sepsis.

Methods: We analyzed data from 516 consecutive adult patients undergoing cardiac surgery at our hospital between 1st January 2005 to 30th April 2006. The patients underwent coronary bypass grafting, valvular surgery or aneurysm surgery. Sepsis was defined as positive blood cultures along with features of systemic inflammatory response syndrome. The rate of sepsis was analyzed in patients receiving $\leqslant$4 units of blood vs >4 units of blood. The correlation of duration of surgery ($\leqslant$5 hours vs >5 hours) and the length of stay in the ICU ($\leqslant$4 days vs >4 days) with the rate of sepsis was also studied.
Results: 2.33% of the 516 patients developed postoperative sepsis associated with positive blood cultures. 40% of the sepsis episodes were associated with Gram negative organisms, 26.7% with Gram positive organisms and 33.3% with candida. Mean blood products used for 516 patients was 5.08 units. The rate of sepsis in patients who received fewer transfusions was 1.27% and those who received 5 or more transfusions was 3.98%, $p = 0.0690$. Mean duration of surgery of 516 patients was 4.90 hours. 0.98% patients with the shorter duration of surgery developed sepsis compared to 6.60% patients with the longer duration, $p = 0.0041$. Mean duration of ICU stay of 516 patients was 4.64 days. 0.82% patients who stayed in the ICU for 4 days or fewer developed sepsis compared to 5.92% patients who stayed in the ICU for more than 4 days, $p = 0.0013$.
Conclusion: In our study, the incidence of postoperative sepsis was higher in patients receiving 5 or more blood units for transfusion, but this was not statistically significant. We also found that that longer duration of surgery and length of stay in ICU has a highly significant positive correlation with incidence of postoperative septicaemia. Studies with larger patient populations need to be done before a positive correlation between blood product transfusion & postoperative septicaemia can be established.

Travel medicine, tropical & parasitic diseases

R2237 A multi-centre retrospective study on intestinal parasitosis in Italy, 2005–2007

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Objectives: Many data on the epidemiology of intestinal parasitosis are available for developing area, not so for industrialised countries. Our aim was to describe the epidemiology of parasitic intestinal infections in Italy, through a multicentre study coordinated by Association of Italian Clinical Microbiologists – Committee of Parasitology.
Methods: We asked twelve laboratories throughout the country to fill a questionnaire about their diagnostic methods and local epidemiology related to the period of 1 January 2005–31 December 2007. The attention was pointed to evaluate the results of standard stool parasitological examination (O&P), cellophane-tape test, Baermann/larvae culture methods and permanent stain (acid fast stain, Giemsa or trichrome stain) for intestinal helminths and protozoa.
Results: All the laboratories performed O&P and cellophane-tape test. 33524 samples were tested by O&P, 337 (1.0%) were positive for helminths and 851 (2.54%) for pathogen Protozoa. Helminths were: *Taenia* spp. 0.35%, *Hymenolepis* spp. 0.18%, *A. lumbricoides* 0.13%, *T. trichiuria* 0.12, *Ancylostoma* spp. 0.08%, *S. mansoni* 0.05%, *D. latum* 0.04% and 851/33524 (2.54%); Protozoa were: *G. lamblia* 2.15%, *E. histolytica/dispar* 0.36% and *I. belli* 0.02%. 2313 samples were examined by cellophane-tape test, 252 (10.89%) were positive for *E. vermicularis*. 7/12 labs used Baermann or larves culture methods for isolation of *S. stercoralis*: 196/14170 were positive (1.38%). 6/12 labs performed Giemsa and 2/12 labs trichrome stain for *D. fragilis*: 378/16357 (2.31%). All laboratories used acid fast stain for *Cryptosporidium* spp.: 44/2647 (1.66%). 4/12 labs used antigen detection for *E. histolytica/dispar*. Only 1 lab cultured suspected cases for *E. histolytica* by Robinson medium: 83/403 (20.6%).
Conclusions: Not all the labs are still organised for good faecal diagnostics and are available to performe adequate data collection. Anyway, faecal parasites and particularly helminths, excluding *E. vermicularis* and *S. stercoralis*, are very rare in Italy. Moreover, more labs should investigate for *D. fragilis* using at least Giemsa stain, and research specifically *S. stercoralis* when risk factors are present. For *E. histolytica* the antigen detection is useful but the gold standard remains the culture that is still available in few labs.

R2238 Increase of imported leishmaniasis in the Netherlands? A 12-year overview (1996–2007)

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Objectives: Surveillance data indicates that the number of cutaneous (CL), mucocutaneous (ML) and visceral leishmaniasis (VL) cases has increased globally during the past decades. Leishmaniasis is not endemic in the Netherlands and is only seen as an imported disease.
Methods: To investigate the trend of the occurrence we analysed all CL, ML and VL patient samples sent to the laboratory at the CIb between 1996 and 2007. We then compared these results to cases reported to the PALGA foundation, a nationwide network and registry of histo- and cytopathology data.
Results: The majority of diagnosed leishmaniasis patients in the Netherlands suffer from CL, and a weak, non-significant increase over the years can be observed. A CL outbreak among Dutch military personnel stationed in endemic regions in 2005 was also noted. ML is rarely found in the Netherlands – we detected only one to three cases a year. However, the occurrence of VL has increased significantly during the last decade in the Netherlands, mainly among patients under 20 years of age.
Conclusion: Physicians in the Netherlands should be aware that leishmaniasis is close to home and can be contracted as close as Southern Europe and that it is not limited to tropical and subtropical regions only.

R2239 Visceral leishmaniasis: report of 6 cases

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Visceral leishmaniasis is considered important zoonotic infection, endemic in Mediterranean countries, caused by the intracellular parasite *Leishmania* spp.
Objective: Analysis of the epidemiological, clinical and laboratory parameters of the disease at the region of responsibility of our hospital, during the last 3 years.
Methods: 4 adults (mean age: 58.7 years) and 2 children (mean age: 1.7 years) were admitted with visceral leishmaniasis. On admission, haematological, biochemical tests and abdominal ultrasound were performed. The parasites were detected by direct microscopy of bone marrow smears. Parasite culture performed in specialised media (NNN and RPMI). Specific IgG antibodies detection by Indirect Immunofluorescence (Vircell microbiologist, Spain), Electrosyneresis, (for detection of active form of the disease) and PCR were also performed.
Results: All adults were in close contact with sray dogs, while one referred recent trip to Pakistan. The incidence of the disease was higher during warm months of the year. Most important clinical features were: fever (100%), hepatosplenomegaly (100%), malaise (50%), flue like syndrome (10%). CRP and liver enzymes were elevated (100%). The most frequent haematologic findings were anaemia (100%), leucopenia–thrombocytopenia (90%) and eosinophilia (10%). The diagnosis was established by detection of amastigotes forms of parasites in bone marrow smears (80%), detection of elevated IgG (>1/400) titers by IFA (90%) and Electrosyneresis (90%). Blood cultures were positive (80%), while PCR was positive in all cases. All patients were treated with liposomial amphotericine B.
Conclusions: 1. Increased incidence in our country is observed during the last years, due to environmental changes and limitation of protective measures against stray dogs. 2. The most common diagnostic approach is the presence of parasites in bone marrow smears and serological

tests. 3. Electrosyneresis and PCR are important for rapid and accurate diagnosis of the disease.

R2240 Therapeutical approach in hydatic cyst disease with multiple location

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Background: Hydatid liver, pulmonary, cerebral and peritoneal disease in children is a serious problem, mainly in areas where the parasite is endemic.
Echinococcosis is a severe disease in childhood inaccessible to an initial radical surgical treatment, medical therapy being an alternative with controversial curative efficacy.
Objective: to evaluate the efficacy of albendazole and to discuss the role of surgery in multiple hidatid cyst disease diagnosed in 29 patients.
Material and Method: The group of study was represented by 14 children aged 2–16 years and 15 adults, mean age 35.4 years, treated in two county hospitals in Constanta between 2000 and 2008.
All patients had multiple hepatic cysts and 12 had coexisting cysts in the lung.
One patient had a peritoneal cyst, 2 had cerebral cysts and 1 had multiple located cysts (liver, brain, lungs and heart).
In 19 cases, medication with albendazole was used as the initial therapy, given as 10 mg/kg daily continuously, for several months (3–9 months).
Results: The overall success (defined as progressive shrinkage and solidification of the cyst) of medical therapy was very low, 1 patient with 2 pulmonary cysts was cured. Age, sex, and the size, location, and number of cysts did not show any relationship to the response to medical therapy. A total of 28 patients (10 primarily, 18 after unsuccessful medical therapy) were treated surgically. During the follow-up period, 3 surgical patients(2 untreated and 1 treated with albendazole), developed recurrent disease.
Conclusion: Medical treatment with albendazole resulted in fewer curative successes than expected; probably a longer period of medical treatment may increase the success rate, coupled with surgery.

R2241 European cluster of imported falciparum malaria from Gambia

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During the comparatively short time period of three months between September and December 2008, TropNetEurop member sites reported 63 patients returning from Gambia with falciparum malaria. The series impressed with a particularly high rate of disease complications and deaths of patients. While the reasons for travel were quite diverse, a striking lack of effective prophylactic measures was apparent in all. Fifty-one patients had not used any malaria chemoprophylaxis. All travellers who indicated that they had taken prophylactic drugs used inadequate or downright wrong ones: two took homeopathic prophylaxis, three used chloroquine only, one used paludrine only, and the remaining stopped taking effective chemoprophylaxis too early. Thus, despite the documented risk of complicated falciparum malaria from Gambia, virtually all patients chose to use no or inadequate prophylaxis. Several were counselled to take this decision by their travel agency, but in a few cases even by their family doctor. The cluster underlines the necessity of competent pre-travel information and adequate protection in travellers, in particular at times when malaria appears to be decreasing but still remains a high risk for non-immune travellers. Although there probably is an overuse of chemoprophylaxis against malaria among tourists travelling to Asia and Latin America, chemoprophylaxis is a must for most travellers to African destinations, and in particular to west Africa.

R2242 The campaign for malaria eradication in Romania, 1923–1963

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Objectives: The present study aims to analyze extensively the history of the eradication campaign against malaria in Romania.
Methods: Retrospective analysis of the Romanian official documents available on malaria epidemiology and eradication campaign.
Results: The median incidence of malaria cases before 1924 was considered to be of about 5,000–6,000 per 100,000 inhabitants. During the period 1925–1940, the median incidence was 879.6 cases per 100,000 inhabitants per year. During the Second World War period (1941–1944) the malaria endemy registered a peak in 1942 with an incidence of 1,218 cases per 100,000 inhabitants. In the following years the incidence increased from 421.5 cases per 100,000 inhabitants in 1944 to 735.1 cases per 100,000 inhabitants in 1946. The Ministry of Health formed a Malaria Commission in February 1947 with the mission to reorganise the fight against malaria based on international guidelines. The efficiency of the complex methods used during the antimalarial campaign begun in 1947, was proved by: 1. the reduction by 99.4% of the new cases and 98.4% of the relapses in the whole country, at the end of 1953, compared with malaria incidence in 1948; 2. 66% decrease of the incidence at the end of 1954 (compared with the year 1953); 3. maintenance of the endemic index at 0 in the zones cleared of malaria and protected against reinfestation by insecticide barriers; 4. haematological control in 86,937 blood samples indicated 351 parasite carriers, out of whom 96.2% *P. vivax*, 3.3% *P. falciparum* and 0.6% *P. malariae*; these results confirmed the efficiency of the methods and the absence of any deficiencies when pulverisation with insecticides was performed in good conditions; 5. no reappearance of epidemic foci among collectivities which continuously and periodically were subjected to "imagocide" protection – with residual insecticides, associated with chemotherapy and, eventually, chemoprophylaxis with synthetic products – during a period of 6 years; there was no sign of acquired resistance in vectors. In 1963, the Romanian authorities informed World Health Organisation about the malaria eradication on its territory.
Conclusion: Malaria was common in Romania until the largely successful campaigns of the twentieth century. Through a combination of vector control strategies and chemotherapy, the disease was gradually brought under control, so that today malaria is not a significant health hazard in Romania.

Resistance and mechanisms of action of antifungals

R2243 Comparative proteomic analysis of fluconazole-resistant and fluconazole-sensitive *Candida glabrata* isolates

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Objectives: The known molecular mechanisms of fluconazole resisitance in *C. glabrata* are not sufficient to explain the resistance in clinical isolates of *C. glabrata*, new resistance-associated genes and other possible underlying resistance mechanisms deserve a great deal of concern and investigation.
Methods: We used proteomics-associated techniques to identify changes in the proteome of fluconazole-resistant isolates of *C. glabrata* compared with fluconazole-sensitive ones in order to identify proteins that are differentially expressed in associated with fluconazole resistance.
Results: Eight proteins were found to be more abundantly represented, and four proteins were found to be less abundantly represented, in fluconazole-resistant strains compared with fluconazole-sensitive ones. These differentially expressed proteins involved in energy metabolism, stress response and macromolecule synthesis.
Conclusion: These results indicate that proteins involved in energy metabolism, stress response and macromolecule synthesis may play a

role in the development of fluconazole resistance in clinical isolates of *C. glabrata*. The study provides further evidence that many different mechanisms are involved in the development of fluconazole resistance in *C. glabrata*. These findings provide scientific basis for discovering new genes and mechanisms associated with fluconazole resistance in *C. glabrata*.

R2244 Susceptibility to fluconazole and voriconazole of aetiologic agents of candidaemia in Saint Petersburg, Russia

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Objective: To study species distribution and prevalence of susceptibility to fluconazole and voriconazole among *Candida* spp., isolated from blood of hospitalised patients in Saint Petersburg.
Methods: Susceptibility testing was performed according to CLSI M44A document.
Results: During years 2003–2008 145 *Candida* spp. strains have been cultured from blood of patients in several tertiary hospitals in Saint Petersburg, Russia and transferred for species identification and susceptibility testing to reference mycological laboratory (Kashkin Research Institute of Medical Mycology).
Species distribution was as follows: *C. albicans* – 36 strains (24.8%), *C. parapsilosis* – 34 (23.4%), *C. guilliermondii* – 28 (19.3%), *C. glabrata* – 14 (9.7%), *C. tropicalis* – 9 (6.2%), *C. krusei* – 5 (3.4%), *C. lusitaniae* – 4 (2.8%), *C. famata* – 2 (1.4%), *C. lipolytica* – 1 (0.7%), *C. dubliniensis* – 1 (0.7%), *C. pelliculosa* – 1 (0.7%), *Candida* sp. – 10 (6.9%).
All isolates were tested for susceptibility to fluconazole. On the whole, 33 (22.8%) strains were resistant (R) or susceptible dose-dependent (SDD) to this drug. All *C. albicans* and all except one *C. parapsilosis* strains were susceptible to fluconazole. All *C. krusei* isolates were R. Low susceptibility (R, SDD) was found in 13 (46.4%) *C. guilliermondii* strains and 12 (85.7%) *C. glabrata* isolates.
Susceptibility to voriconazole was tested in 56 *Candida* spp. strains, isolated from blood during 2006–2008. On the whole, 8 (14.3%) isolates were R or showed intermediate (I) susceptibility to this antifungal. Among strains with low susceptibility (R, I) to voriconazole species distribution was as follows: *C. guilliermondii* – 3 strains, *C. krusei* – 2, *C. glabrata* – 2, *C. parapsilosis* – 1.
Conclusions: 1. *Candida* non-*albicans* species caused 75.2% of cases of candidaemia in Saint Petersburg. 2 Among aetiologic agents of candidaemia prevalence of susceptibility to fluconazole was 77.2% and to voriconazole – 85.7%.

R2245 Virulence factors and susceptibility patterns of *Candida* species isolated from patients with obstructive uropathy and bladder cancer

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Objectives: to study the distribution of virulence factors among candida species isolated from patients with candiduria and their association with resistance to antifungal agents.
Methods: urine specimens from 250 patients divided into 3 groups were examined: group1 (n = 50) cancer bladder, group2 (n = 100) obstructive uropathy and group 3 (n = 100) simple recurrent urinary tract infection (UTI). *Candida* isolates were identified by CHROM-agar and by Candifast Es Twin test. Resistance to antifungal agents was tested by disc diffusion test for fluconazole (FCZ 25Ug; Mast, UK) and E test strips for fluconazole, voriconazole and amphotericin B (AB Biodisk Solna, Sweden) using Sabaroud dextrose agar. Biofilm formation (BF) was detected by tube and spectrophotometric plate adherence methods and quantitated by crystal violet staining and XTT reduction assays. Phospholipase activity (PLA) was screened using Sabouraud egg yolk agar and proteinase (SAP) was detected using bovine serum albumin agar.
Results: candiduria was detected in 24% of patients with highest incidence among obstructive uropathy patients (67.2%). *Candida non-albicans* species (tropicalis, krusei and glabrata) were significantly higher than *C. albicans* (73.1% versus 26.8%; $p < 0.001$) in this group. BF, SAP and PLA were detected in 39.3%, 44.3% and 72.1% of isolates respectively with significant production among group 2 patients. The tube adherence and crystal violet staining methods were simple sensitive methods for detection and quantitation of BF. *C. krusei* and *C. tropicalis* were the highest biofilm-producing species (81.8% and 63.6%). FCZ showed poor activity against all isolated candida species (24%-32.8%) with high MIC values (>256 ug/ml). Voriconazole was active against all isolated candida species with low MIC values (0.064–1 ug/ml) except in *C. glabrata* (22%). All isolated candida species were more sensitive to VCZ compared to FCZ; particularly *C. krusei* isolates (90% versus 9%). AMB remained to be an effective drug with absolute sensitivity and low MIC in *C. glabrata* isolates.
Conclusion: BF, PLA and SAP production are important virulence traits in candiduria causing UTI. BF is common in non-*albicans* species particularly *C. krusei* and *C. tropicalis* in patients with obstructive uropathies. Resisance to FCZ is highly associated with virulence traits so it may no longer be the drug of choice for treatment of candiduria. AMB or VCZ are better alternatives while *C. glabrata* is best treated by AMB.

Fungal infections

R2246 Rapid identification of *Candida* species from clinical specimens by RFLP-PCR method

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Nowadays, opportunistic infections have been increasing as a result of *Candida* species and non-*albicans*. Indeed traditional methods were used for identification clinical isolates of *Candida* spp are time-consuming and also they are not appropriate for rapid and accurate reliable identification.
Objective: Using universal, ITSI and ITS4, in this study, we could amplify ITSI-5.8S-ITSII regions at both 80 clinical sample and 3 standard isolates. Clinical strains were isolated from urine, lip, throat and cheek of patients of oncological ward of Imam hospital of Sari in Iran.
Method: Chromagar was used for preliminary detection of candida species. DNA from the selected specimens was extracted by Glass-Bead strategy, and PCR-RFLP methods were done on the extracted genomes.
Result: Furthermore, we successfully identified all isolated species using tow restriction enzyme, *C. albicans* (77.5%) was the most common species among them. Consequently, *C. glabrata* (15%), *C. tropicalis* (5%), *C. krusei* (2.5%) respectively. Although applied primers and enzyme were able to identified *C. parapsilosis*, *C. guilliermondi*, *C. dubliniensis*, there were no such isolates among all identified isolates.
Conclusion: RFLP-PCR using ITSI and ITS4 primers and restriction enzyme is rapid, easy, reliable and also applicable method in clinical laboratory for identification of medically important *Candida* species.

R2247 Fungal strains isolated from liver transplant recipients

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Introduction: Fungal infections in liver transplant recipients constitute constantly increase therapeutic problems. *Candida* genus is the leading fungal pathogen in transplant medicine combine with high risk of morbidity and mortality in liver transplant patients.
Aim of study: The aim of our study was to analyze of fungal strains isolated from samples taken from clinical materials of liver transplant recipients hospitalised in Department of General and Liver Surgery Central Clinical Hospital in Warsaw in 2007.
Material and Methods: The total number of 50 positive clinical samples from liver recipients were examinated, which constituted 39%

of positive materials from the Transplant Department: clinical samples were taken from respiratory tract – 30 (59%), wound swabs – 6 (12%), urine samples – 5 (10%), drain tips – 5 (10%), other materials – 5 (10%). The specimens were inoculated into Sabouraud's medium with chloramphenicol and gentamicin (bioMerieux). The isolation and identification of the isolates was performed with the use of CHROMAgar (Becton Dickinson) and biochemical test API ID32C (bioMerieux).
Results: The collection of fungal isolates included 62 strains, yeastlike fungi 58 strains (94%) and moulds 4 strains (6%). The most often isolated strains from *Candida* genus were: *C. albicans* – 31 (50%), *C. glabrata* – 9 (15%), *C. inconspicua* – 8 (13%), *C. parapsilosis* – 5 (8%). Other yeastlike fungi constituted 8% – 5 strains. In total 4 strains of *Aspergillus fumigatus* (6%) were identified. Clinical samples taken from respiratory tracts were positive in 60%, 37 fungal strais were isolated from this material (*C. albicans* n = 19 (51%)).
Conclusions:
1. The most common fungal pathogens isolated from liver transplant recipients was *C. albicans* – 31 strains (50%).
2. 17 isolates (28%) were natural resistance to fluconazole – primary regimens for yeast infections.
3. *Aspergillus fumigatus* isolates were cultured in 6% of the total number of fungal strains.

R2248 Fatal cutaneous zygomycosis from *Saksenaea vasiformis* in a young patient after a car accident. First isolation in Greece

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Introduction: *Saksenaea vasiformis* is a rare human pathogen with a world-wide distribution associated with soil and invasive lesions after traumatic implantation. We report a fatal case of cutaneous zygomycosis caused by *S. vasiformis* in a previously healthy man suffered soil contaminated wounds in a car accident.
Case report: A 30-year old patient was admitted to our hospital due to mild cranial contusion. Three days after the accident at the left posterior thoracic and lumbar region a rapidly progressive necrotising lesion appeared. The patient became febrile (38.5–39ºC), while was heamodynamical stable. He underwent extensively surgical debridment and 2 specimens were taken for culture and histopathologic examination. Exudates and necrotic tissue crude preparations were examined by microscopy. On Gram staining, Gram(+) andGram(−) rods were observed, while on direct microscopy, wide, non-septate, hyaline, typical for Zygomycetes hyphae were present. Conventional culture revealed mixed bacterial flora. Minimally manipulated tissue samples were cultured in Sabouraud dextrose agar at 25, 30 and 37ºC.At all temperatures, woolly, rapidly growing, zygomycotic colonies appeared in 24 hs and covered the entire Petri dish in 48 hs. Because the isolate didn't sporulate in malt extract and potato dextrose agars, it was cultured in Czapek's, 1% water and in saline agar at 25, 30 and 37ºC but remained sterile for over 2 months. The isolate was identified by sequencing the Internal Transcribed Spacer region (ITS) after DNA extraction from pure culture and ITS PCR amplification using the primers ITS1and ITS4. The derived sequence was compared to published fungal sequences and showed more than 99% homology with those published for *S. vasiformis*. In vitro susceptibility testing to antifungals (CLSI, Etest methods) was not possible to perform, probably due to the very fragile nature of the non-sporulating isolates' hyphae. Despite of the combination of the aggressive surgical debridement of the infected tissues and the systemic treatment with amphotericin B and posaconasole, the patient developed extensively destructive necrotising local invasion and died from septic shock on the 13th day of his hospitalisation.
Conclusion: *S. vasiformis* is an emerging human pathogen, that is most often associated with cutaneous lesions after trauma that could lead to a fatal outcome. Laboratory identification may be difficult or delayed because of the mould's failure to sporulate on the primary isolation media.

AIDS and HIV infection

R2249 A significantly different dysmetabolic profile of the two available non-nucleoside reverse transcriptase inhibitors: nevirapine versus efavirenz

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Introduction: Altered metabolism represents an emerging feature of HIV-infected patients (p) treated with combined antiretroviral therapy (cART), but a different profile seems to regard the two available non-nucleoside reverse transcriptase inhibitors (NNRTI): efavirenz (E) and nevirapine (N).
Methods: Among over 1,100 p treated with cART for >12 months, the metabolic pattern of NNRTI was assessed according to three different backgrounds. The first one included antiretroviral-naive p starting a NNRTI-based regimen; the second included a large spectrum of p experienced with 2 to 10 therapeutic lines (but still NNRTI-naive); the third group included p who added for the first time a NNRTI only on late rescue therapies with at least 4 drugs (and including protease inhibitors).
Results: 441 p treated with E were compared with 378 p taking N in our prospective observational survey lasting 12 to 44 months, by a multivariate analysis of serum lipid-glucose levels, and other metabolic abnormalities. Among the 241 p naive to antiretrovirals, an altered triglyceridaemia was more common ($p < 0.001$) in the E versus the N group. Considering the 386 antiretroviral-experienced p who introduced a NNRTI for the first time, the frequency of hypertriglyceridaemia appeared greater in the E group ($p < 0.0001$), with earlier development of this feature in p on E versus N ($p < 0.0001$). Also in the 192 p receiving salvage HAART, the rate of hypertriglyceridaemia-hypercholesterolaemia-hyperglycaemia tested greater among p treated with E versus N ($p < 0.03$ to $p < 0.001$), and the time to peak alterations was more rapid in the E group ($p < 0.03$). Comparing the 441 p receiving E with the 378 p on N, the frequency of elevated triglyceride, cholesterol, and glucose levels was greater in E-treated p ($p < 0.0001$ to <0.005). Some grade of lipodystrophy was present in 311 pre-treated p, but an appreciable ameliorement occurred after NNRTI introduction in 21 p of the E group, versus with 63 p on N ($p < 0.002$).
Conclusions: A sufficiently prolonged follow-up shows that E may not resolve dysmetabolism, but might also prompt metabolic abnormalities with more frequency and intensity compared with N. The two available NNRTI have a significantly different dysmetabolic profile, which leads to an increased interest in prospective pathogenetic and preventive investigations.

R2250 Switch from lopinavir-ritonavir towards atazanavir-based combinations in patients with sustained virological-immunological control, and predominant dysmetabolic abnormalities

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Introduction: Among the long-term untoward effects of antiretroviral therapy, metabolic abnormalities are the most frequent, and are burdened by relevant risks to prompt major vascular events. The metabolic syndrome, and especially hypertriglyceridaemia and hypercholesterolaemia are a major concern during protease inhibitor (PI)-based regimens, where lopinavir/ritonavir (L/r) represents a standard of care for its potency-durability, whereas the more recent PI atazanavir (A) is expected to show a reduced metabolic toxicity, although literature reports are to date scarce.
Patients and Methods: In an observational, prospective, case-control (1:2) study involving our cohort of >1000 patients (p), among 162 p treated with 2 NRTI and L/r since >12 mo and with undetectable viraemia since >6 mo, who developed hypertrigyceridaemia and/or hypercholesterolaemia, 54 p (33.3%) switched to A/r (39 p) or unboosted A (15 p), and were compared with the remaining 108 p who continued L/r. For this interim analysis, p who completed >12 mo of follow-up were evaluable.

Results: Although the mean initial levels of triglyceridaemia and cholesterolaemia were significantly greater in the p group switched to A ($p < 0.01$ and $p < 0.04$, respectively), just these p experienced a significantly higher drop of serum lipid levels during the 12-mo follow-up: -91.2 ± 53.8 mg/dL for triglyceridaemia ($p < 0.003$), and -33.6 ± 26.9 mg/dL for cholesterolaemia ($p < 0.01$), versus the L/r control group. Even more favourable figures were recognised among the 15 p treated with unboosted A (−143.2 mg/dL for triglyceridaemia, −56.7 mg/dL for cholesterolaemia), but statistical assessment was not feasible. During the 12-mo follow-up, no virological-immunological failure occurred. The substitution of L/r with A/r or A contributed to maintain the stable virological-immunological response already reached under L/r-based HAART, while significant metabolic advantages were observed in p switched to A/r and A alone, when considering total triglyceridaemia and cholesterolaemia, from the 6th to the 12th mo of follow-up. No significant benefits were observed with regard to the HDL-LDL cholesterol fractions.
Conclusions: Further studies are needed to assess the role of A/r and A also in PI-naive p, taking into consideration the role of the different nucleos(t)idic backbone, and examining extensive p samples followed for a longer time, with in-depth laboratory and instrumental monitoring.

R2251 HIV disease and management and pancreatic damage. Epidemiology, clinical issues, pathogenetic correlates, and therapeutic indications

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Introduction: The frequency, risk factors, and clinical-therapeutic features of pancreatic anomalies were assessed in a prospective case-control study.
Methods: 1081 HIV-infected patients (p) followed for >12 months were evaluated.
Results: The 435 p (40.2%) who experienced >1 episode of pancreatic laboratory abnormality had a longer duration of seropositivity, exposure to protease inhibitors, a more frequent CD4 count <200 cells/μL, AIDS, chronic liver-biliary disease, and hypertriglyceridaemia, while no relation was found with antiretroviral administration, and the duration or type of administered NNRTI, when compared with the 646 controls, who never developed pancreatic anomalies. Among the above-mentioned 435 p, elevated-prolonged laboratory alterations eventually associated with signs of organ involvement occurred in 166 p (38.2%), and were related to the administration of ddI, d4T, 3TC, pentamidine, cotrimoxazole, or anti-TB/anti-mycobacterial therapy, cytotoxic chemotherapy, substance-alcohol abuse, opportunistic infections, chronic liver-biliary disease, a protease inhibitor-based HAART, and hypertriglyceridaemia. However, no difference was noticed between the 46 p with clinical-imaging evidence of pancreatic involvement and the remaining 120 asymptomatic p, when assessed according to the same risk factors. Although recurrences of enzyme alterations involved 69.6% of overall p, in only 30.1% of cases a change of the antiretroviral-antimicrobial therapy became necessary. An acute but uncomplicated pancreatitis occurred in 9/46 symptomatic p (19.6%). A 2–4-week gabexate and/or octreotide administration (performed in 79/166 cases: 47.6%), achieved a significant laboratory, clinical, and imaging cure-improvement in 82.3% of cases, with a better success rate of combined (gabexate mesilate+octreotide) versus single (gabexate mesilate or ocreotide) therapy. A significantly reduced tendency to disease recurrences, and a better tolerability of antiretrovirals, were also noticed.
Conclusions: Epidemiological and pathogenetic studies are strongly needed to assess the evolution of pancreatic abnormalities during the HAART era. The indications to gabexate mesilate-octreotide administration deserve further investigation in the HIV disease setting.

R2252 The phenomenon of "AIDS Presenters". Opportunistic infections associated to a late, first diagnosis of AIDS, after a decade of HAART availability

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Introduction: Notwithstanding the availability of HAART, AIDS notifications continue to occur, with increasing prevalence for patients (p) who missed-neglected their condition.
Patients and Methods: All cases of AIDS notified since the year 2001 were compared with those found in the decade preceding HAART availability (1986–1995).
Results: Compared with the pre-HAART era, a significant drop of frequency of overall AIDS cases occurred: from a mean 58.3 ± 11.2 patients-year observed in the decade 1986–1995, to 14.9 ± 6.2 patients-year during years 2001–2006 ($p < 0.001$), together with an increased mean age ($p < 0.002$), female gender ($p < 0.01$), sexual vs i.v. transmission ($p < 0.001$), and proportion of immigrant vs resident p ($p < 0.03$). In the HAART era, the most evident drop of frequency interested opportunistic diseases linked to a CD4 count below 50–100 cells/μL, while a proportional rise of tuberculosis, pneumonia, lymphomas, and other neoplasms was observed. The frequency of both *Candida* oesophagitis and *Pneumocystis carinii* pneumonia remained stable, as the first 2 AIDS-related conditions. After HAART availability, the following diagnoses were neurotoxoplasmosis, wasting syndrome, AIDS-dementia complex, and non-Hodgkin's lymphomas. P with multiple AIDS-defining diseases, and also AIDS diagnoses made at death, even showed a paradoxically increased frequency and absolute number during the HAART era vs the prior decade ($p < 0.001$ and $p < 0.02$), while no difference was found as to HIV-associated immunodeficiency. Surprisingly, an underlying anti-HIV therapy was a more common event until 1995, vs p observed in the HAART era ($p < 0.001$), since during recent years AIDS notification tends to be increasingly associated with the first diagnosis of HIV infection.
Discussion: When facing p with opportunism, clinicians should maintain an elevated suspect for an advanced (but missed-untreated) HIV disease. A continued attention will help a more rapid recognition and an appropriate management of p who could not take benefit from HAART, since they remained unaware of their disease, or refused controls and treatment.

R2253 HIV infection, HAART, and gynaecomastia. Epidemiological, clinical, and pathogenetic relationship

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Introduction: Gynaecomastia (G) is an emerging untoward event in patients treated with HAART.
Patients and Methods: Through a cross-sectional study performed on around 1000 HIV-infected patients (p) treated with antiretrovirals at our reference centre in Bologna (Italy), we identified all cases of G related to the administration of at least 12 consecutive months of HAART, to assess possible correlations of G with a spectrum of clinical, laboratory, and therapeutic variables (and including all adverse effects of HAART itself). All p with true G (as distinguished from lipomastia by an ultrasonography assay) were considered evaluable, while p with other predisposing conditions (endocrine disease, alcohol abuse, liver cirrhosis, and use of drug possibly predisposing to G), were carefully excluded.
Results: Twenty-one out of 616 evaluable HIV-infected male p (3.4% of our p population), developed a true G when aged 12–58 years. Seven p out of 21 never received protease inhibitor (PI)-containing therapies, while efavirenz-based regimens apparently prompted G in 7 p who were naive for PI, and worsened this disturbance in three further p who abandoned PI for efavirenz. Considering nucleoside analogues (NA), two p developed G during treatment conducted with dual isolated NA.Comparing the different administered NA, stavudine seemed to be the most commonly used compound, also taken for the longest time ($p < 0.01$). A complete hormonal workup did not detect significant abnormalities, save in one p, who had slight serum FHS,

LH, and testosteron abnormalities (with normal prolactin levels). When considering the eventual correlation with the most common HAART-induced disturbances, some forms of lipodystrophy was concurrent in all the 21 p with G, while hypertriglyceridaemia, hypercholesterolaemia, and hyperglycaemia were found in 15, 9,and 3 p, respectively. During the subsequent 12–36-month follow-up, a spontaneous ameliorement of G was never observed, notwithstanding eventual HAART modifications. Due to local hypersthesia, tenderness, and discomfort, two p resorted to surgery.

Conclusions: G is probably an underestimated problem in the setting of HAART. The frequent association of G with other HAART-related dysmetabolism suggests possible common pathogenetic causes.

R2254 A novel, reliable laboratory technique to assess hypersensitivity to all available antiretroviral compounds: the cellular antigen stimulation test

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Introduction: Recently, novel allergometric techniques allow to test inhalants, food, and also drugs by specific in vitro assays. A flow cytometry technique based on the search of sulphidoleukotrienes LCT4, LTD4 and LTE4 released by basophils stimulated in vitro by IL-3 in presence of the examined antigens (cellular antigen stimulation test, or CAST), has a 80–90% sensitivity-specificity rate, and becomes particularly useful when prick tests are not applicable, and allergic reactions are not mediated by allergen-specific IgE immunoglobulins.

Methods and Results: During the past three years, 18 HIV-infected subjects (11 females and 7 males, aged 32–52 years), underwent a standardised, specific CAST assay, due to serious cutaneous (8 cases), systemic (4 patients), and combined cutaneous-systemic hypersensitivity reactions (6 subjects), apparently not elicited by the introduction of abacavir and nevirapine (which are the antiretroviral agents burdened by the greatest frequency of expected early allergic reactions, mediated by already recognized pathogenetic mechanisms). Based on the results of CAST testing, an allergic intolerance to ritonavir (6 cases), lopinavir, nelfinavir, and didanosine (three cases each), saquinavir and lamivudine (two cases), and fosamprenavir, zidovudine, zalcitabine, stavudine, and efavirenz (one case each), was documented: in 15 cases out of 18 (83.3%) multiple intolerances were detected. A perfect relationship was documented between the results of CAST testing and the panel of combined antiretroviral compounds recently experienced by each allergic patient, and a CAST-based elimination of in vitro allergenic molecules allowed a rapid introduction of another effective antiretroviral combination.

Conclusions: Adverse events to antiretroviral drugs are quite frequent among HIV-infected patients, compared with the general population. Further, controlled studies are strongly needed to implement in vitro allergometric testing in patients treated for HIV infection, who are exposed to unpredictable drug intolerance reactions. In fact, HIV-infected subjects may suffer from frequent allergic drug reactions which may be difficult to be systematically recognized (due to the frequent, multiple concurrent pharmacotherapy, and the combined antiretroviral therapy itself).

R2255 Increasing survival in HIV patients with HLA-B57 allele: report and analysis of a Spanish series

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Objectives: Different evolution of the human immunodeficiency virus (HIV) in different patients has suggested the influence of an immunologic factor that explains such differences. In the last few years HIV-1 infected long-term non-progressors patients have been broadly studied. Among this special group a high prevalence of haplotype HLA-B57 has been detected and its influence in the HIV-1 replication capacity still remains unclear.

Methods: All patients on HIV infection who carried the HLA-B57 allele were selected for this study. Epidemiological, clinical, immunological and therapeutic findings were analysed. The survival was measured in years free of disease.

Results: 8 HIV-1 patients were studied. All of them had heterocigotic haplotype HLA class I with the HLA-B57 allele. Patients were 6 women and 2 men with a mean age at the time of diagnosis of 26.5 years (19–36 y). All of them were spanish except one rumanian. 50% of cases had heterosexual transmission. The mean CD4 at the time of diagnosis was 466 cell/micrl. The mean HIV-RNA viral load at the time of diagnosis was 6,882 copies/mL. High Active Antiretroviral Therapy (HAART) was started only in 2 patients. 75% of patients did not need start HAART having a mean survival of 11.5 years (7–18 y) free of disease.

Conclusion: The HLA-B57 allele in HIV-1 infected patients is associated with a long-term survival measured in years free of disease. The reason that this allele produces a slower progression of the infection remains unclear. Future research focused on the influence of this allele in the HIV infection may lead to a better understanding of the disease and a design of future therapeutic targets.

Hepatitis

R2256 The cytokine profile in peripheral blood cells from chronic hepatitis C virus (HCV)-infected patients: effects of pegylated interferon and ribavirin

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Objectives: Hepatitis C virus (HCV) is a major cause of chronic liver disease that leads to cirrhosis. Natural immune responses, both cellular and humoral, are not capable of terminating HCV infection in most patients. Host immunity involves effector mechanisms networked by its elaborated cytokines.

The study was design to assess serum level and supernatant in peripheral blood mononuclear cells (PBMC) in patients with chronic hepatitis C (CHC) infection on the background histopathologic changes.

Methods: The study included 14 cases CHC with cirrhosis. All patients were treatment of pegylated interferon and ribavirin. Blood samples were collected from all subjects at 0, 2, 4 and 12 weeks at the treatment.

Serum pro- and anti-inflammatory cytokines levels were assayed by flow cytometer using BDTMCytometric Bead Array (CBA) Human Inflammation Kit.

The cytokines in supernatant from PBMC were detected by ELISA using the BD Biosciences kit.

Moreover in serum were assayed sFas by ELISA method.

The activity of chronic hepatitis C was scored according to the degree of hepatic process inflammation and determined 1, 2 or 3.

HCV genotyping was performed by commercial test Inno-Lipa HCV. The Level of HCV RNA in sera was evaluated by Real Time PCR method.

Results:

1. The decrease of viraemia after 12 week's therapy in all patients was observed.
2. In 8 cases virus HCV RNA was not detected. The decrease of viraemia correlated with decrease ALT.
3. The level of sFas was many times higher in CHC patients in comparison with healthy persons.
4. There was observed higher level all measuring cytokines pro-inflammatory before treatment only in this patients who were elimination HCV.
5. HCV RNA concentration in sera were between 2.32×10^4–3.45×10^6 copies.
6. Amongst the 14 patients HCV genotype 1a was found in one patient and genotype 1b in 13 patients.

Conclusions:

1. The results suggest that higher level of pro-inflammatory cytokines is connected with elimination HCV.

2. The higher sFas expression in CHC patients than healthy, may suggest mechanism of liver injury caused by disorder apoptosis.

R2257 Recognition of hepatitis C virus in a type of drug that is abused by intravenous drug users in Iran

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Introduction: Hepatitis C virus (HCV) has now been recognized as a major health problem worldwide and estimated number of infected people is over 170 million people worldwide. Many studies in Iran are reported the intravenous drug users (IDUs) had HCV infection at least between 38% and 47% and in recent several studies higher percentages were reported. HCV can be easily transmitted through blood products and infected syringes, and infection rates are typically high among injecting drug users (IDUs). NORJIZAC (Also known as "handmade temgizac", "Ab Crack (Crack solution") is a slang name of a drug that abused by numbers of Injecting drug users in Iran since 5 years ego. Main Route of using NORJIZAC is via intravenous. In spite of high prevalence of HCV among IDUs, specially NORJIZAC users, many of them haven't given any history of sharing in needles and syringes during injections.
Material and Methods: In a cross sectional study, 14 NORJIZAC vials that were bought from smugglers in different times and different locations within a period of 4 months, were tested for hepatitis C virus. RNA extraction and then C-DNA synthesis by using reverse-transcriptase enzyme and after that Real time PCR with specific primer and probe with tagman probe method, were made on norgizac. All laboratory staff were unaware about the nature of the drug.
Results: 2 of 14 vials were positive for hepatitis C virus with acceptable viral load more than 10000/ml.
Discussion: HCV is estimated to be about 10 times more infectious than HIV per unit of blood required, and therefore, requires less exposure than HIV to reach high prevalence.
Although significant advances have been made in preventing HIV infection among IDUs with harm reduction programs, both prevalence and incidence of hepatitis C remains high among IDUs. One of the most important point about the environmental survival of HCV is prolonged staying of virus in the environment.
Conclusion: Formerly we thought HCV was transmitted during needles and syringes sharing among the injecting drug users, but we had several patients without history of sharing. Results of this study show that NORJIZAC itself could be infected with hepatitis C virus and avoiding of sharing in injections couldn't be enough against accepting HCV during drug using, maybe this event could be occurred in another country.

Virology non-HIV/non-hepatitis

R2258 Seoul hantavirus infection in the far east of Russia

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It is generally known that *Rattus norvegicus* (brown rat), widely distributed around the world, is the natural reservoir of hantavirus Seoul (SEOV). In Russia first cases of HFRS related to SEOV were recorded in 1989 in far-eastern city Vladivostok (Primorsky region) with morbidity 0.3 cases per 100 000. Annual number of HFRS cases caused by SEOV in far-eastern Russia has increased during the past decade: from 1.6 cases per 100,000 in 1992 to 6.0 per 100,000 in 2000.
Objective: Investigation of SEOV infection and the cause of increased morbidity in the south of far-eastern Russia.
Methods: serology, virology and genetic study.
Results: Overall 344 cases of SEOV infection were officially registered during last decade. Average prevalence of hantavirus infection in population of *R. norvegicus* in seaport Vladivostok varied from 4.3% (1992) to 33.8% (2001). The considerable increase of HFRS morbidity with seasonal peak in spring was related to acute phase of epizootic process of SEOV infection in *R. norvegicus* and prevalence of infected rodents with specific antigen and low avidity antibodies. Clinical course of HFRS associated with SEOV was characterised by dominance of mild, moderate and atypical forms of infections (87%). During past ten years we observed the increase of severe forms of SEOV infection from 5.9% to 22.0% including three fatalities. Genetic analysis showed that different forms of HFRS were associated with strain Vladivostok (VDV) of SEOV circulating in Vladivostok city. Strain VDV is closely related to SEOV strains from Cambodia. The primary cause of SEOV infection activity in urban foci is the increase of *R. norvegicus* population. Decrease of measures of disinfestations in large seaport resulted in stable active circulation of VDV strains in different urban facilities.
Conclusion: There is active urban focus of hantavirus infection associated with circulation of SEOV in *R. norvegicus* population in the south of far-eastern Russia. For effective prevention of hantavirus infection we propose annual large-scale complex of disinfestations and disinfections measures.

R2259 Evaluation of measles-specific immunity among healthcare workers in a northern Greek hospital

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Objectives: Measles is an acute, highly contagious viral disease affecting people of all ages. While it is primarily a disease affecting children, it may also affect adults, among whom several complications such as pneumonia and encephalitis are common. Hospital staff incurs a high risk of infection and CDC guidelines recommend that non-immune persons should be vaccinated. Since November 2005 an increase in adult cases of measles has been reported in Greece and this rise was mostly detected in northern Greece. Our objective was to evaluate immune status against measles virus among healthcare workers of a northern Greek hospital and the subsequent vaccination of non-immune hospital staff.
Methods: Serum samples were taken from 144 healthcare workers, including physicians and nurses, who work in different hospital departments. 44 of the employees were male and 100 female and their ages ranged between 28 and 50 years. Samples were tested for the detection of specific measles IgG antibodies by using an indirect ELISA assay. All subjects were asked if they had had measles or been vaccinated against it in the past.
Results: 141 of the 144 subjects (97.9%) had detectable immunity against measles. 120 of the immune subjects (83%) had high titers of protective antibodies, while 21 subjects (17%) had low titers. The majority of those with high immune titers (85/120) reported measles infection in the past history, whereas the majority of those with low immune titers (16/21) reported past vaccination.
Conclusions: High rates of immunity to measles virus were detected among our hospital personnel, either due to natural immunity or due to vaccination. The highest titers of protective antibodies were observed among those infected by measles in the past. Vaccination was recommended for the three non-immune healthcare employees. Despite the high immunity rates that were observed, continual alertness as well as vaccination of non-immune personnel are regarded as essential due to the rising adult cases of measles and the consequential severe complications.

R2260 Comparison of CMV IgG avidity assay on IMMULITE 2000 versus VIDAS on clinically derived serum samples

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Objectives: The avidity index (AI) of CMV IgG antibodies can be used to differentiate between primary CMV infection and past infections as a complementary test besides the CMV IgM antibody assay. The presence of CMV IgM antibodies is sometimes very difficult to interpret, because it can still be detected a long time after the primary infection.
The present study shows the results obtained with the IMMULITE 2000 compared with the well established VIDAS CMV IgG avidity assay.
Methods: The IMMULITE 2000 CMV IgG avidity assay is a three-cycle immunoassay utilising a CMV antigen-coated 1/4-inch bead as the solid phase for the capture of human anti-CMV antibodies and an alkaline phosphatase-conjugated anti human IgG monoclonal antibody

for detection. A dissociation reagent is then added to elute the weakly bound IgG while Tris-based buffer is added as the baseline. Following the addition of an enzyme antibody conjugate for signal detection, paired results obtained on each sample are used to calculate the avidity index. Samples used for evaluating assay performance are 167 patient serum specimens procured from our hospital and from general practitioners.

Results: A method comparison between the IMMULITE 2000 and the bioMérieux VIDAS CMV IgG avidity assays using 167 clinical samples yielded an overall agreement of 74.3% (124/167) between IMMULITE 2000 and VIDAS results for high- and low avidity. AI >0.8 was defined as high avidity and AI <0.2 as low avidity for the VIDAS assay per its package insert. For IMMULITE 2000, AI >0.8 was categorised as high avidity an AI <0.4 as low avidity. The IMMULITE 2000 CMV IgG avidity assay demonstrated a relative sensitivity of 96% (66/69) and a relative specificity of 95% (18/19). Among these 167 clinical samples 50 sera were IgM positive. In table 1 the CMV IgG avidity results are shown.

CMV-IgG avidity testing in 50 CMV-IgM positive sera

Immulite 2000	VIDAS			
	High	Gray zone	Low	**Total**
High	13	3	0	16
Gray zone	1	14	0	15
Low	0	7	12	19
Total	14	24	12	50

In 16 CMV IgM dubious positive serum samples in both systems no low avidity IgG was found. In 94% of the 101 IgG positive and IgM negative serum samples no low avidity IgG could be detected in both systems.

Conclusion: The IMMULITE 2000 gave in 70% a clear result in AI as support of the CMV IgM interpretation, while the VIDAS system scored only 52 percent.

The advantage of the IMMULITE 2000 system above the VIDAS system is a fully automated high-throughput immunoanalyzer.

R2261 Comparison of the diagnostic value of ZEBRA IgM assays versus VCA IgM assays in determining the infection status of EBV-infection

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Objectives: In general IgM antibodies are predominantly produced in the acute phase of infectious diseases. Detection of IgM antibodies against EBV VCA-antigen is widely used. VCA IgM antibodies can also be detected in (long) past EBV-infections. ZEBRA IgM antibody detection is a new commercial available assay used in EBV-serology

This study was undertaken for a general evaluation of two ZEBRA IgM assays to get more information about their usefulness in determining the status of EBV-infection, especially in the acute phase of the infection.

Methods: Twelve consecutive sera were gathered from 4 patients in different stages of the EBV-infection. From these 4 patients the haematological picture was also known

Forty-eight patient sera were collected from our routine serum bank, representing all profiles of EBV-results based on DiaSorin's EBNA-IgG, EA-IgG and VCA-IgM ETI-assays.

The clinical status related to all sera was further determined with Mikrogen EBV recomLine IgG (including IgG-avidity). The VCA IgM results were also obtained by performing Mikrogen EBV recomLine IgM blots. The ZEBRA IgM results were obtained using Mikrogen EBV recomLine IgM blots and the Clindia EBV ZEBRA IgM Elisa test.

Results: The results of the 12 consecutive sera from the 4 patients were showing that VCA IgM-antibodies are persisting longer then ZEBRA IgM. These findings were confirmed with another 48 sera. The positive rate of the ZEBRA IgM assays (Mikrogen 19/25 – Clindia 21/25) in the active stages of the infection was almost as high as the DiaSorin VCA IgM assay (23/25), but more negative results were obtained in stages with no active EBV-infection. In relation to the Clindia EBV ZEBRA IgM test (21), the Mikrogen IgM blot (19) was less sensitive for detecting ZEBRA IgM antibodies, but more specific. In relation to the DiaSorin VCA IgM assay (41), the Mikrogen IgM blot (7) showed a very low sensitivity in detecting VCA IgM-antibodies, but showed an excellent specificity.

Conclusion: Both ZEBRA IgM assays showed more reliable results in detecting active stages of EBV-infection then the DiaSorin VCA IgM assay. The predictive values for an active EBV infection using both ZEBRA IgM assays are comparable with the DiaSorin VCA IgM assay. The predictive values of both ZEBRA IgM assays in non-active stages of infection however, are much higher then the DiaSorin VCA IgM assay. The Mikrogen EBV IgM blot showed a high specificity but a poor sensitivity for detecting VCA IgM antibodies.

Categories	Conclusions	VCA IgM		Zebra IgM	
		Dia Sorin EIA pos	Mikrogen blot pos	Clindia EBV EIA pos	Mikrogen blot pos
Never been infected	5	1	0	1 dub	0
Very early infection	5	5	0	4	3
Early infection	4	4	0	3	3
Active infection	14	12	7	12 + 1 dub	12
Reconvalescent stadium	2	2	0	2	1
Past infection	30	17	0	5 + 2 dub	3
Total	60	41	7	26 + 4 dub	22

R2262 Atypical appearance and course of progressive multifocal leukoencephalopathy as the first clue of a missed HIV infection. Advanced MRI-spectroscopy functional-metabolic imaging

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Introduction: Progressive multifocal leukoencephalopathy (PML) is still an underinvestigated HIV-associated CNS opportunism. An exceptional case report characterised by motor involvement, occurred as the 1st AIDS-defining event in the absence of appreciable immunodeficiency in a patient (p) with a missed HIV infection, and was assessed by associated functional-metabolic MRI techniques (spectroscopy-MRI).

Case report: A 45-y-old p had HIV infection detected after the appearance-worsening of predominantly motor abnormalities (dysarthria, coordination anomalies, ophthalmoparesis), in absence of other signs-symptoms. HIV infection was found in absence of an appreciable immunodeficiency (as expressed by a CD4 count of 564 cells/μL), and viral load was limited to 24000 HIV-RNA copies/mL. An extremely potent combination antiretroviral therapy was immediately started with 3TC, abacavir, and lopinavir/R, with subsequent adjunct of efavirenz and enfuvirtide. Virological studies of cerebrospinal fluid (CSF) disclosed elevated levels of JC virus (11668 copies/μL) so that a diagnosis of PML was confirmed, after observing consistent neuroradiological findings at CT-MRI scans. Despite the aggressive therapy approach, which achieved undetectable HIV viraemia, a CD4 count >700 cells/μL, and disappearance of JCV after 1 mo, the neurological picture rapidly deteriorated from a predominantly motor level (severe dysphagia, trunk-limbs paresis, sphincter anomalies), while cognitive impairments never occurred. The unfavourable clinical evolution paralleled the neuroradiological worsening, and our p rapidly deceased 5 months after the diagnosis, due to respiratory insufficiency. A combined MRI-spectroscopy study performed 1 mo before death included a proton (1H) spectroscopy, and a MRI study-calculation of water diffusion and anisotropy:through this novel technique combining morphological-metabolic findings, multiple abnormalities involving the subtentorial white substance were detected (the encephalic trunk and ponto-bulbar structures), which usually are not part of PML course.

Discussion: A JCV-associated PML may occur as the first AIDS-defining event in p unaware of HIV infection, and may rapidly evolve with atypical clinical features (motor deficits largely prevailing over cognitive ones), even with an initial CD4 count >500 cells/μL, and a quick decay

of both HIV-JCV viral loads. The neuroradiological features of our p were implemented by ultraspecialistic spectroscopy-MRI techniques, which have no reported equivalents until now in the available literature

R2263 Neurophysiciatric changes in associated with human parvovirus B 19 meningoencephalitis

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Human parvovirus B 19 (B19) is a common infectious pathogen in humans. Infection with B19 has been associated with various neurological syndromes including encephalitis, seizures, disturbance of consciousness, meningitis, cerebellar ataxia, transverse myelitis, optic neuropathy, neurologic amytrophy, Guillain–Barré Syndrome, paraesthesia and carpal tunnel syndrome. In two retrospective studies, B19 virus was detected in 4.6% and 4.3% of undiagnosed meningoencephalitis cases.

Since its discovery, B19 has been linked with a broad spectrum of clinical syndromes. An aetiological role for the virus has been confirmed in erythema infectiosum, transient aplastic crisis, persistent infection manifesting as pure red cell aplasia in immunocompromised persons, non-immune hydrops fetalis and arthritis. Less commonly recognized, but receiving increasing attention, are the neurological manifestations, a variety of which have been described in patients with either clinically diagnosed or laboratory confirmed B19 infection.

A 75-year-old, previously healthy and an intellectual retired doctor was admitted to hospital due to abrupt beginning of headache, confusion, agitation, dysarthria and fever. Physical examination revealed abnormal mentation, cognitive disorders, dysarthria and apparent neck stiffness. The initial fever was around 38ºC and resolved in two days.

Patients with viral meningoencephalitis usually have signs and symptoms of meningeal inflammation, but, in addition to headache, fever, and nuchal rigidity, it is characterised by alterations of consciousness. Our patient was diagnosed as B19 meningoencephalitis with the inflammatory CNS profile, abnormal mentation, cognitive disorders, dysarthria and apparent neck stiffness combined with the microbiological data. Generally, the neurophysiciatric changes were seen in childhood B19 meningoencephalitis. To the author's knowledge, our case differs from other adult cases reported in the literature due to dominant presenting pattern of cerebral involvement with cognitive disorders, personality changes and dysarthria. Moreover, the level of Parvovirüs B19 DNA was relatively low, which resemble to offer a subacute or chronic character

The patient we present is a rare case of B19-associated with neurophysiciatric disorders in which mortality seems to be high and that B19 might be the responsible agent for CNS infections particularly in those without specific diagnosis.

Mycobacterial infections (including diagnosis)

R2264 Frequency of tuberculosis in Qeshm, the biggest island in the Persian Gulf

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Objectives: Despite availability antituberculosis drugs for almost 50 years, tuberculosis (TB) continues to exert an enormous toll on world health. The incidence of TB is increasing all over the world. Qeshm represents a region in south of Iran that is the biggest island in the Persian gulf with 23 thousands inhabitants with a long tradition in TB control, including a centralisation of the bacteriological diagnostic facility. The present study was intended to analyze the transmission of *Mycobacterium tuberculosis* by a combination of conventional epidemiological approaches.

Methods: *Mycobacterium tuberculosis* analyzed in this study were collected at the Health Care Center in Qeshm, Iran. A total of 81 new, bacteriologically verified TB cases were registered in Qeshm Island between 2003 and 2008. All the isolates were examined for their susceptibility to ethambutol, isoniazid, streptomycin, rifampin, and pyrazinamide by using a radiometric culture system (BACTEC). The data obtained from the cultures analyses were interpreted by using demographic data, such as age, sex, ethnicity, and residence, for the patients. The risk factors among the patients for being part of an active chain of transmission, as opposed to demonstrating reactivation of a previously acquired latent infection, were estimated by statistical analyses (SPSS).

Results: A total of 81 clinical isolates belonging to patients having pulmonary and extra pulmonary tuberculosis were collected during Jan 2003 to Nov 2008. The incidence of tuberculosis in female was 25.9% and in male was 74.1%. This survey observed 47.1% of immigrated Afghans and 39.1% of Pakistanis were infected with tuberculosis. Regarding the literacy 57% were unlettered. 91.7% of people referring to health centre were new patients. 68.8% people were infected with pulmonary tuberculosis. The peoples over 60 year were highest group infected to pulmonary tuberculosis (30.4%) and age groups 30–44 were highest the cases infection external pulmonary tuberculosis. The major chains of recent transmission were localised to distinct geographical regions in the area.

Conclusion: TB is frequent among immigrants, especially from Afghanistan and Pakistan, but it is apparently readily suspected, diagnosed, and treated by the health care system. Indigenous patients with pulmonary symptoms are not primarily suspected to have TB and, therefore, play an important role in recent TB transmission in Qeshm.

R2265 Extensively drug-resistant tuberculosis in patients immigrated from Eastern Europe. Microbiological, therapeutic, and public health issues

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Introduction: Extensively Drug Resistant (XDR) tuberculosis (TB) is a worldwide emergency. The increased number of patients (p) immigrating from countries where the health care assistance could not ensure adequate drug delivery-monitoring is a major concern in Europe.

Methods-Results: During the 2nd half of y 2006 and the 1st half of y 2007 a 30-y-old male from Moldova and a 24-y-old female from Ukraine underwent very prolonged hospitalisations due to XDR TB. The first p, with TB known since 6 y developed XDR due to frequent treatment discontinuations. On the ground of in vitro supplementary sensitivity assays, cycloserine, para-aminosalycilic acid, capreomycin, ethionamide, and linezolid were added, obtaining clinical-microbiological cure after 9 mo of hospitalisation. Three mo after discharge, our p maintained an effective 6-drug regimen on Day-Hospital basis, but 3 mo later another 5-mo admission was needed after retrieval of a positive sputum. An outpatient treatment was conducted on Day-Hospital basis for 3 mo, but positive sputum prompted a 3rd admission lasting since 3 mo. Our 2nd p who came to Italy with a XDR TB, had an unfavourable course, and was tested in vitro for 2nd-choice drugs, which suggested a cycloserine, para-aminosalycilic acid, capreomycin, ethionamide, and moxifloxacin adjunct, and achieved clinical-bacteriological cure and hospital discharge after 5 mo, despite a concurrent chronic hepatitis C which hampered liver tolerability. During the subsequent 3-mo Day-Hospital follow-up, a 5-drug association ensured a temporary cure, but 4 mo later another 3-mo admission was needed due to repeated positive sputum searches.

Conclusions: The management of the emerging XDR TB encompasses elevated clinical suspicion, diagnostic accuracy, availability of susceptibility assays of 2nd-3rd line drugs, adequate isolation, and public health issues, when prolonged hospitalisations or protected discharges are needed. The frequent involvement of foreign immigrants is burdened by further social-economic, cultural, and administrative problems. The easy development of life-threatening and contagious XDR TB in health care contexts where low-cost anti-TB drugs are not always available, is in contrast with the huge danger and the incredibly elevated costs of these episodes which need prolonged hospitalisation-isolation, and

enormous technologic and health care efforts. A systematic planning of the most adequate management-prophylactic measures aimed at containing-preventing XDR TB in the next future, is strongly needed.

R2266 First-line anti-tuberculosis drug susceptibility rates of *Mycobacterium tuberculosis* clinical isolates over a 6-year period

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Pathogens of the *Mycobacterium tuberculosis* complex, remain one of the leading killers worldwide. In Europe, a large number of multidrug-resistant (MDR) and more recently, extremely drug-resistant (XDR) tuberculosis cases have been reported.
Aim: The assessment of resistance to first line anti TB drugs of *M. tuberculosis* isolates in a TB Unit of a tertiary General Hospital
Methods: During the last 6 years period (2002–2007), 23,709 clinical samples have been cultured in solid (Lowenstein-Jensen) and liquid (MGIT 960 Becton Dickinson) growth media. 317 of these samples, one for each of the involved patients, revealed *M. tuberculosis* complex mycobacteria. The identification has been made by molecular methods – GenoType *Mycobacterium* MTBC/CM/AS (Hain Lifescience) – and the susceptibility testing has performed in the non-radiometric system MGIT 960 (bioMerieux). Drugs used were: Streptomycin (STR 1.0 μg/ml), Isoniazid (INH 0.1 μg/ml), Rifampicin (RIF 1.0 μg/ml), Ethambutol (EMB 5.0 μg/ml) and Pyrazinamide (PYZ 100 μg/ml). The turn-around time found to be 9–12 days. Among the 317 involved patients 259 were Greeks and 60 immigrants, 220 men and 99 women.
Results: Antimycobacterial susceptibilities of the 317 *M. tuberculosis* complex isolates, showed that 75.07% of these isolates were susceptible to all five first line anti-TB-drugs, whereas 24.93% were resistant at least to one drug. More specifically the resistance rate to streptomycin was 16.09%, to isoniazid 16.40%, to rifampicin 2.84%, to ethambutol 2.84% and to pyrazinamide 1.58%, while 5.04% of isolates were multidrug-resistant (MDR).
Conclusions: 1. In our study, Resistant tuberculosis seems to be in an acceptable level, with the resistance to first line anti TB drugs in a descending trend, comparing to former results of ours. 2. The use of MGIT 960 for testing first-line drugs streamlines the drug susceptibility testing process. 3. Delay in recognition of drug resistant cases, influences negatively the initiation of effective therapy and the co sequencing control of Tuberculosis.

R2267 Clinical and epidemiological features of tuberculous meningo-encephalitis paediatric cases over 10 years at a teaching clinical hospital in Romania

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Objective: Meningo-encephalitis caused by *Mycobacterium tuberculosis* still holds an important place among the central nervous system infections and represents a relevant part of the extrapulmonary localisation of this infection, by its diagnostic and therapeutic challenges.
Method: The patients were recorded by the personal data concerning age, sex, geographic provenance, epidemiological data regarding heredocollateral and their personal history regarding mycobacterial infection, clinical stage at presentation, time interval until the diagnosis and until starting tuberculostatic therapy. Cerebrospinal fluid samples were examined in dynamic before and during the therapy, using direct light microscopy with Ziehl-Nielsen coloration and solid cultures with Loewenstein-Jensen culture media. The imagistic evaluation (CT, MRI) was also applied. We have evaluated the clinical evolution, complication implying central nervous system and those associated with the tuberculostatic therapy. We overviewed the antituberculous therapy, and the resistance profile.
Results: The group was formed by 35 non HIV-infected paediatric patients aged from 18 months to 17 years, admitted during a period of 10 years in a teaching Clinical Hospital (1997 october-2008 october). 54.2% were boys and 45.8% were girls, originated from four districts in the western side of the country, 40% from the urban and 60% from the rural area. The range of the symptoms at presentation was very wide, from headache and fever, associated meningeal irritation signs to coma, cranial nerve paresis. The average time span until establishing the diagnosis and the begin of the correct treatment was 15 days. 88.5% of the patients were previously treated with different classes of antibiotics. 25.7% of the cases were complicated with hydrocephalia and we have confronted with toxic hepatitis related to the therapy in 14.2% of the patients. In 3 of the cases the strains were resistant to one or two of the major therapeutic agents. 74.3% of the patients fully recovered, while 25.7% remained with sequelae like hydrocephalia, blindness or paresis.
Conclusion: The physician should mantain a high degree of suspicion regarding this disease because any delay in commencing the right treatment is associated with a significantly more reserved outcome and important sequelae.

R2268 Whole-blood and cerebrospinal fluid interferon-gamma release assay for diagnosis of tuberculous meningitis

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Objectives: The interferon-gamma enzyme-linked immunosorbent assay QuantiFERON-TB Gold (QTFG) has been designed for use with whole blood and has been studied mainly for diagnosing active or latent pulmonary tuberculosis. We aimed to assess the accuracy of the commercially available QTFG in blood and adapted variants of the assay using cerebrospinal fluid (CSF) for the diagnosis of tuberculous meningitis and compare it with the results of conventional diagnostic tools.
Material and Method: We prospectively studied 33 patients with tuberculous meningitis diagnosed and treated in the Infectious Diseases Hospital from Iasi, Romania during the last 2 years. A lot of 33 patients with bacterial or viral meningitis and without known BK exposure were used as negative controls.
Results: A bacteriological confirmation (direct exam from the CSF or Lowenstein-Jensen culture) of the diagnosis was possible in only 21.2%. The accuracy of the QTFG assay from the CSF was higher than from blood (65.6% vs 60.6% sensitivity, 96.7% vs 91.2% specificity) or compared with the tuberculin skin test (sensitivity 54.5%, specificity 71.4%). The positive predictive value of a positive QTFG test from the CSF was 95.2%. The test's performance was not significantly influenced by patient's age (5 to 76 years), immune status (6 patients with AIDS) or prior anti-tuberculous treatment.
Conclusion: Despite its low sensitivity (in whole blood or CSF), due to its high specificity, the QuantiFERON-TB Gold test could be an important tool for the early diagnosis of tuberculous meningitis.

R2269 QuantiFERON TB Gold assay in tuberculosis diagnosis in patients from areas with high and low TB incidence

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Objective: Evaluation of Quantiferon TB Gold in Tube method (QFT) for the latent TB diagnosis in patients originated from countries with high (group A) and low (group B) incidence of TB.
Material: 948 whole blood samples from 153 (16.1%) individuals belonging to group A and 795 (83.9%) to group B.
Method: Performance of Quantiferon TB Gold in Tube (Cellestis, Australia) method according to the manufacturers' instructions.
Results: QFT positive results was detected in 93/153 (60.8%) individuals of group A and 273/795 (29.35) of group B ($p < 0.0001$). Clinical information for previous BCG vaccination was available in 351 cases: 65 of group A and 286 of group B, where QFT confirmed latent TB in 36/65 (50.8%) and 46/286 (16.1%) of group B ($p < 0.0001$). As it was expected, Tuberculin Skin Test (TST) was positive in all 351 cases with previous BCG vaccination.

Conclusion: QFT is a highly diagnostic and useful method, especially in patients originated from areas with high incidence of TB. In contrast to widely used TST, it reduces over diagnosis of latent TB in previously BCG vaccinated.

R2270 Increasing the sensitivity of acid-fast bacilli detection by using concentration technique: an Indian study

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Objectives: To increase the sensitivity of reporting of Acid fast *Bacillus* (AFB) in the given specimen by using concentration technique besides direct method of smear examination, for all the specimens received for AFB screening, in a tertiary care hospital in India.
Methods: All specimens with a request for Acid fast bacillus (AFB) stain were subjected to both direct Zeiehl-Neelson (ZN) technique and concentration technique (National tuberculosis guidelines).
Direct smear method: smears made from the sputum/specimen samples were heat fixed and stained using the ZN technique for AFB staining.
Concentration method for AFB staining of sputum (National Tuberculosis Institute guidelines)
For 4–5 ml of sputum/specimen, double the volume of 4% of NaOH is added aseptically and mixed well. The bottle is placed on a shaker and kept in 37ºC incubator for 20 minutes. Then, sterile distilled water added approximately 20 to 30 ml, mixed well and centrifuged. The sediment is used to make smears; heat fixed and stained using the ZN technique for AFB staining.
Results: Total number of specimens received between Jan 2007 and Dec 2008 were about 1600. Sterile fluids included Pericardial. Pleural, peritoneal, ascitic, vertebral, synovial, cerebrospinal fluids and blood. Tissue specimens included endometrial, synovial, visceral pleura and lymph nodes. All patients tested positive by concentration method were treated with standard anti tubercular treatment. Table 1 explains the AFB positivity in direct and concentration methods.

Table 1. AFB positive smears by direct and concentration techniques of various specimens

S No	Specimens	Total numbers	AFB positives			Percentage of false negative reporting
			Total	by direct method	by concentration	
1	Sputum/BAL	1058	158	85	73	46
3	Sterile fluids	143	8	5	3	37.7
4	Urine	142	14	8	6	42.5
5	Pus	69	7	6	1	14.3
6	Tissue	46	4	3	1	25

Conclusion: In India, two people become sputum-positive for tuberculosis every minute. One sputum-positive patient can infect 10.15 individuals a year. The Revised National Tuberculosis Control Programme (RNTCP) aims to stop the spread of disease. Failure to detect persons with infectious TB will continue to spread infection in the community. By adapting direct method alone, nearly 50% of the samples would have been missed, thereby missing out on patients who needed treatment. Hence, increasing the sensitivity of AFB smear detection by concentration technique should be an additional method besides direct smear examination.

R2271 AIDS-associated atypical mycobacteriosis other than *Mycobacterium avium-intracellulare*: a 14-year survey of *Mycobacterium xenopi* and *Mycobacterium kansasii*

R. Manfredi, L. Calza (Bologna, IT)*

Introduction: A prompt and effective diagnosis and a timely treatment of atypical mycobacteriosis and especially *Mycobacterium kansasii* and *Mycobacterium xenopi* disease, remains a serious challenge for clinicians engaged in the management of the immunocompromised host, including HIV disease.

Patients and Methods: Sixteen and eleven HIV-infected patients with a microbiologically-confirmed *M. kansasii* and *M. xenopi* respiratory infection respectively, have been observed in a 14-year period, out of over 4,100 hospitalisations performed because of HIV-associated disorders. These episodes were carefully evaluated from an epidemiological, bacteriological, clinical, and therapeutic point of view.
Results: In 12 out of 27 cases (44.4%) a bacteraemia was also retrieved. The proportionally reduced crude frequency of atypical mycobacteriosis as HIV-related complication, which virtually disappeared after introduction of potent antiretroviral combinations (HAART) in 1996, is underlined. In early nineties, the lack of effective antiretroviral regimens made frequent the association of this opportunism with full-blown AIDS, a mean CD4+ lymphocyte count of around 20 cells/μL, and an extremely variable chest X-ray features. The recent detection of two further episodes was due to a late recognition of a far advanced HIV disease (so-called AIDS presenters), complicated by multiple opportunistic disorders.
Discussion: *M. kansasii* and *M. xenopi* respiratory and/or disseminated infection continues to occur, and pose relevant diagnostic problems, including late or missed identification due to slow culture and frequently concurrent opportunistic disease. Serious therapeutic difficulties, due to the unpredictable in vitro antimicrobial susceptibility profile of these organisms, and the need to start as soon as possible an effective combination therapy which should not interfere with other medications (especially HAART), are also discussed.

Infection in the immunocompromised host and transplant recipients

R2272 Lower respiratory infection in cancer patients

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Objectives: The epidemiology of infections in cancer patients undergoes changes which impact empiric therapy. The aim of this study was to describe the type and the microbiology of lower respiratory infection occurring in hospitalised cancer patients.
Methods: Retrospective review of medical records over a two years period (2007–8). Classic culture methods, Api system for microbial identification and CLSI breakpoints for antimicrobial susceptibility were used.
Results: During this period 558 sputum specimens were sent from 389 patients and 36 microbiological documented infections were recorded. The 36 patients were febrile (>38ºC) immunosuppressed, under chemotherapy. 25/36 patients had an underlying solid organ cancer (18 with lung cancer) whereas 11/36 had a haematologic malignancy. The mean time of hospitalisation until the infection has been occurred was 9 days. 30/36 patients had monomicrobial infection (83%). Bacteriological analysis revealed: 13 strains of MRSA, 12 *P. aeruginosa*, 7 *K. pneumoniae*, 4 *Candida* sp, 3 *E. cloacae* and 3 *A. baumannii*. 11/36 patients (38%) occurred bacteraemia due to the same phenotypic strain and 4 of these patients died.
Conclusion: The predominant site of lower respiratory infection in our patients was microbiologically documented pneumonia (9.2%). The principal pathogens were Gram negative bacteria with high rate of resistance. Thus in cancer patients the empiric therapy should be based on local microbiologic and susceptibility data.

R2273 Cytomegalovirus infections in liver transplant recipients

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Background and Objective: Cytomegalovirus (CMV) infections are the single most common viral infection since successful liver transplantation. The incidence of cytomegalovirus infections ranges 8–19% in seropositive liver transplant recipients (CMV R+). However it

is vital that the centres have their own infection data profile. We aimed to find out viral infection incidence in liver transplant patients in DEU Hospital (Izmir/Turkey) in order to make a decision strategy for antiviral prophylaxis or preemptive therapy.
Methods: Between January 2003 and December 2007, 212 cases of liver transplantation were performed at our centre. Records of case were examined for risk factors of CMV infection retrospectively. Age, gender, MELD score, renal insufficiency, cold ischaemia time, bacterial and fungal infection, immunosuppressive therapy, massive blood transfusion, vascular and hepatic artery thrombosis, rejection were accepted as independent risk factors. None of the patients have received prophylaxis or preemptive treatment for CMV.
Results: In this study 99 (46, 7%) out of 212 patients were consulted to infectious disease and clinical microbiology specialists because of post-transplant infections and 20 (9, 4%) patients required antiviral treatment. Of 212 recipient, only 10 (4, 7%) were CMV seronegative. Of 20 patients used antiviral treatment, 12 (5, 7%) had Herpes simplex virus I/ Varicella zoster virus infection and 8 (3, 7%) had CMV infection. Even there was no microbiological evidence, clinical response was determined with antiviral therapy in two cases with CMV infection. The details of six cases proven as CMV diseases are presented in table. All of these CMV cases had CMV R+ and were followed up by CMV antigenaemia assay. In the absence of effective antiviral prophylaxis or preemptive therapy, CMV infections usually occurred three months to six month following transplant surgery.
Conclusion: Upon analysis of viral infections our centre, the incidence of viral infections in liver transplant patients was found to be lower than expected and CMV diseases usually were the type of delayed-onset. According to these findings antiviral prophylaxis after transplantation is not recommended for the recipients in Dokuz Eylul University Hospital. However preemptive therapy should be used for those liver transplant recipient having high risk factors for CMV disease.

Table: The details of six cases proven CMV diseases

	Case I	Case II	Case III	Case IV	Case V	Case VI
Primary diseases	Hepatitis B	Alcoholism	Hepatitis C	Hepatitis B	Hepatitis B + minimal change disease	Hepatitis C
Age/gender	66/M	59/M	58/F	53/M	41/F	58/M
MELD score	15	19	14	16	20	18
Transplant type	Cadaveric	Living	Living	Cadaveric	Living	Living
Renal insufficiency	–	–	–	–	Renal transplantation	–
Cold ischaemia time (min)	370	120	90	330	120	90
Bacterial and fungal infection	+	+	–	–	–	–
Immunosuppression	Tacrolimus Prednisolone MMF	Tacrolimus Prednisolone MMF	Ciclosporin Prednisolone MMF	Tacrolimus Prednisolone MMF	Tacrolimus Prednisolone MMF	Ciclosporin Prednisolone MMF
Massive blood transfusion	16	6	4	4	7	20
Vascular and hepatic artery thrombosis	–	+	–	–	–	–
Rejection	–	–	–	–	–	–
Date of CMV infection determined	Sixth month	Third month	Third month	First month	Fourth month	Third month
Prognosis	Exitus	Alive	Alive HCV nuks	Alive	Alive	Alive HCV nuks

R2274 Asymptomatic *Toxoplasma gondii* infection in liver transplant patients

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Objectives: *Toxoplasma gondii* infection in transplant recipients can lead to toxoplasmosis, which in some cases may have a rapid disease course or to be fatal. Serological tests do not often contribute to the diagnosis. Although PCR may early identify the infection, this technique is not well standardised and there is no consensus on the optimal protocol to be used in laboratories. We homogenised our PCR data with those of a Real-time PCR targeting different *T. gondii* genes.
Methods: *T. gondii* genes were explored before and after liver transplantation (LT) with PCR and Real-time PCR (LightCycler, Roche) targeting bradyzoite (BAG-1) matrix (MAG-1), cyst surface (SAG-4) and dense granule (GRA-6) specific genes other than B1 gene. These have been developed by us and retrospectively evaluated in PBMC specimens from 7 LT patients in whom serological status (anti *T. gondii* IgG) before transplantation was known in 4 patients only.
Results: We found 4 PCR positive specimens of which 2 with B1 gene (after LT) and 2 with GRA-6 and BAG-1 (before LT). With LightCycler (expressed as *T. gondii* genome equivalents/ml) the overall positivity rates were 12 before and 17 after LT. In particular, in marked contrast with PCR results, the number of positive specimens before LT were 4 (B1), 2 (SAG-1), 3 (SAG-4 and MAG-1, respectively). After LT we did detect 7 (B1), 3 (SAG-1), 5 (SAG-4) 3 (MAG-1). Two patients were found to have elevated DNA copy number of SAG-4 in post-LT specimens only.
Conclusions: Transmission of toxoplasna infection via LT is extremely uncommon with only few confirmed cases previously reported. LightCycler based method can identify a relatively high incidence of potential new infections compared with PCR excluding most of the false-negative PCR results associated with contamination with previously amplified products. This is of greater use for monitoring LT recipients with negative serological tests at risk of developing toxoplasmosis and managing therapy. LT patients did not receive TMP/SMX as post-transplant infection prophylaxis. Improvement and standardisation of diagnostic tests, especially real-time PCR targeting markers expressed on bradyzoites or matrix is also important in order to prevent relapses and symptomatic disease.

R2275 Comparative evaluation of daptomycin and pharmakocinetically-guided vancomycin therapy in patients with prolonged neutropenia or haematopoietic stem cell transplantation

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Objectives: Limited data on the use of daptomycin, which exhibits extended activity against Gram-positive bacteria, in immunocompromised patients (pts) are available.
Methods: We retrospectively studied pts treated with daptomycin during prolonged neutropenia or after haematopoietic stem cell transplantation (HSCT). Results were compared to a control group of pts with pharmakocinetically-guided vancomycin therapy (target trough level <15 mg/l).
Results: Overall, we identified 15 pts with daptomycin and 18 pts with vancomycin therapy (median age 53 and 58 y). Both agents were always added to a broad-spectrum antibacterial regime, including antifungal therapy in 11 pts. Underlying conditions were HSCT in 10 and 11 pts, haematological malignancies in 4 and 7 pts and solid tumour in 1 and none pt with daptomycin and vancomycin therapy, respectively. Eighteen pts had neutropenia, 15 pts had immunosuppressive comedications (steroids, cyclosporine, mycophenolate mofetil) and 9 pts had graft-versus-host disease. Pts had fever of unknown origin (28), urinary tract infections (3), soft tissue infection (1) or pneumonia (1). Six pts with daptomycin therapy have had vancomycin pretreatment.
With start of daptomycin and vancomycin the concurrent antibiotic regime was modified in 6/15 and 8/18 pts, respectively ($p = 1$). The median daily doses were 4 mg/kg (range 2–9) for daptomycin and 22 mg/kg (range: 13–36) for vancomycin. The median durations of daptomycin and vancomycin therapy were 8 (range 1–20) and 10 (range 3–21) days, respectively.
Defervescence was achieved in 9/15 and 13/18 pts treated with daptomycin and vancomycin, respectively ($p = 0.49$). The median ratios of the serum creatinine at the end of therapy compared to baseline were 1.15 and 1.12 for daptomycin and vancomycin ($p = 0.5$). Except for nausea (2) and skin rash (1) in 3 vancomycin treated pts, no other toxicicities attributable to either drug were observed.
Conclusion: Daptomycin showed similar efficacy and tolerability compared to pharmacokinetically-guided vancomycin therapy in this series of immunocompromised pts. Further studies are needed to explore the role of daptomycin in this particular group of pts.

Community-acquired infections including CAP, sepsis, STD, . . .

R2276 Survivin: any potential implication in early sepsis?

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Objectives: Sepsis is initiated by leukocyte and endothelial activation, followed by massive cytokine surge, activation of inflammation and coagulation. Survivin, a protein initially described as an inhibitor of apoptosis is intensely expressed in tumour cells while in normal adult, differentiated tissues its expression is absent. Previous in vitro studies indicated that extracellular survivin up-regulates adhesion molecules on the surface of leukocytes changing their reactivity pattern, suggesting immunoregulatory properties for extracellular survivin. Given our previous observations, we aimed to study the potential involvement of circulating survivin in the pathogenesis of sepsis.
Methods: In 31 septic patients we analyzed serum levels of survivin over the first 7 days upon admission. Fourteen healthy volunteers served as controls.
Results: Patients (24 males/7 females) had the median age of 60. The primary infection had urinary (12), respiratory (14), abdominal (3), dental (1) and cutaneous (1) origin. Five patients had concomitant known malignancies. Five patients (16%) died during the first week. Survivin was detected in nine (29%) patients, ranging between 13.5–812 pg/ml. There was no correlation between survivin levels, severity of sepsis or outcome. Circulating survivin was undetectable in volunteers while all cancer patients had high survivin levels.
Conclusion: Sepsis does not lead to circulating survivin. However, detectable survivin may reveal concomitant comorbidities that may indirectly aggravate the prognosis.

R2277 Inflammatory systemic response and aetiology in community-acquired pneumonia

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Objectives: The aim of this study was to evaluate the systemic cytokine profile (tumour necrosis factor alpha (TNF), interleukin (IL) 1, IL6, IL8 and IL10), C reactive protein (CRP) and procalcitonin (PCT) in patients with community acquired pneumonia (CAP), according to their aetiological agent.
Methods: A prospective study was performed in 685 hospitalised patients with CAP from two tertiary hospitals. Cytokines, PCT and CRP measurements were obtained on day 1. Data were analyzed with the SPSS 15.0 software package.
Results: Data are presented as median and interquartile range in Table 1. Statistically significant differences are marked with an asterisk.

Table 1. Inflammatory cytokines and markers in the most important CAP aetiological agents

Day 1	Virus (n = 12)	Entb (n = 13)	Strpn (n = 118)	Pseae (n = 18)	Myc/Chla (n = 22)	*Legionella* (n = 24)
RCP* mg/dl	12 (8.9–16.8)	20.1 (12.55–31.45)	19.85 (10.3–28.4)	10 (7.4–13.8)	11.3 (7.5–24.3)	24.9 (21.3–33.5)
PCT* ng/ml	0.24 (0.16–0.47)	1.585 (0.56–8.965)	1.71 (0.48–7.37)	0.44 (0.14–0.62)	0.19 (0.09–0.48)	0.71 (0.5–3.15)
TNF-α* pg/ml	14 (10–27)	14.5 (11.5–68)	27 (16–47)	23 (15–42)	25 (21–40)	49 (40–72)
IL-1 pg/ml	13 (4–14)	20 (8.5–41)	16 (4–30)	16 (4–29)	24 (5–35)	19 (5–35)
IL-6* pg/ml	66 (30–192)	168.5 (58–339.5)	144 (38–305)	105 (22–223)	40 (21–79)	202 (69–1548)
IL-10 pg/ml	22 (1–27)	6 (0–25.5)	7 (0–21)	6 (3–12)	9 (0–15)	3 (0–12)
IL-8* pg/ml	4 (3–9)	54.5 (13.5–79.5)	6 (2–19)	12 (8–20)	12 (5–22)	16 (11–35)

Entb, Enterobacteriaceae; Strpn, *Streptococcus pneumoniae*; Pseae, *Pseudomonas aeruginosa*; Myc/Chla, *Mycoplasma/Chlamydia*. *$p < 0.05$.

Conclusions: Viruses had a higher IL-10 median while Enterobacteriaceae, *Legionella*, and *S. pneumoniae* showed higher levels of IL 6. PCT is lower in CAP caused by viruses, *Mycoplasma* or *Chlamydia pneumoniae*. *Legionella* displayed the highest levels of RCP, TNF and IL-6, while Enterobacteriaceae had the highest level of IL-8.

R2278 Accuracy of clinical presentation in predicting the aetiology of acute bacterial meningitis

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Objective: To determine whether acute bacterial meningitis (ABM) aetiologies can be differentiated based on clinical and biological data available on admission.
Methods: prospective observational study of adults hospitalised with ABM at a university hospital in Spain over a 10-year period (1997–2006). Among the 78 cases we included 71, corresponding to the most common microorganisms causing ABM: *S. pneumoniae* (33), *N. meningitidis* (19) and *L. monocytogenes* (19). We analyzed demographic data, predisposing factors, clinical features and laboratory data on hospital admission.
Results: Clinical and biological data are shown on Table 1. In the univariate analysis, the following variables were associated with pneumococcal meningitis: history of head trauma or cerebrospinal fluid (CSF) leakage (sensitivity "S" 30.3%, specificity "Sp" 100%, positive predictive value "PPV" 100%, negative predictive value "NPV" 61%), previous meningitis episode (S 18.2%, Sp 100%, PPV 100%, NPV 57.1%). The presence of petechiae was associated with meningococcal ABM (S 44.4%, Sp 95.9%, PPV 80%, NPV 82.5%). Age >60 (S 77.8, Sp 67.3, PPV 45.2, NPV 89.7), clear CSF (S 61.5, Sp 89.1, PPV 61.5, NPV 89.1), lymphocyte predominance in CSF (S 36.4%, Sp 95.7%, PPV 66.7%, NPV 80%), CSF protein <5 g/L (S 87.5%, Sp 48.9%, PPV 37.8%, NPV 91.7%), and CSF Gram's stain without microorganisms (S 57.9%, Sp 82.7%, PPV 55%, NPV 84.3%) were associated with *Listeria*. In the multivariate analysis, *Listeria* ABM was associated with clear CSF, lymphocyte predominance, and CSF Gram's stain without microogranisms; *S. pneumoniae* with history of head trauma or CSF leakage and *N. meningitidis* with the presence of petechiae.

Table 1. Clinical and biological data by aetiologies

	S. pneumoniae N (%)	*N. meningitidis* N (%)	*L. monocytogenes* N (%)	p
Data available on admission				
Underlying diseases				
Head trauma/CSF leakage	**10/33 (30.2)**	0/18 (0)	0/18 (0)	0.002**
Previous meningitis	**6/33 (18.2)**	0/18 (0)	0/18 (0)	0.028*
Immunocompromised	7/33 (21.2)	3/18 (16.7)	**11/18 (61.1)**	0.004
Age >60 years	10/33 (30.3)	7/19 (36.8)	**14/18 (77.8)**	0.04*
Clinical manifestations				
Petechial rash	1/30 (3.3)	8/18 **(44.4)**	1/18 (5.6)	<0.001**
CSF				
Clear	4/25 (16)	1/17 (5.9)	8/13 **(61.6)**	<0.001**
Lymphocytic	2/30 (6.7)	0/16 (0)	4/15 **(26.7)**	0.03**
Protein <5 g/L	16/28 (57.1)	7/17 (41.2)	14/16 **(87.5)**	0.02**
Gram's stain	25/33 (75.8)	18/19 **(94.7)**	8/19 (42.1)	0.001**
Other microbiological test				
CSF culture	27/33 (81.8)	12/19 (63.2)	18/19 **(94.7)**	
CSF PCR	11/11 (100)	7/7 (100)	1/4 (25)	
Blood cultures	27/33 (81.8)	6/19 (31.6)	15/18 **(83.3)**	
Blood PCR	4/11 (36.4)	5/7 **(71.4)**	0/2 (0)	

**$p < 0.05$ in multivariate analysis; *$p = 0.05$–0.1 in multivariate analysis.

Conclusions: A history of head trauma or CSF leakage and a previous meningitis episode are highly predictive of pneumococcal meningitis. The presence of petechiae is suggestive of meningococcal origin. A CSF with lymphocyte predominance is highly predictive of *Listeria* ABM. However, globally the clinical features lack of sensitivity for establishing the aetiology of acute bacterial meningitis. CSF Gram's stain is the best tool available on admission for the diagnose of ABM, and when it doesn't show microorganisms it's suggestive of *Listeria* ABM.

R2279 The CURB-65 criteria in bacteraemic community-acquired pneumonia patients

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Objectives: CURB-65 is the key procedure in evaluation of patient condition at admittance. In this retrospective study we aimed to evaluate the usefulness of CURB-65 criteria in admitted patients with positive hemoculture. We analyzed the predictive value of CURB-65 criteria in average duration of hospital stay and mortality.
Methods: We collected data regarding age, sex, bacteria isolated from blood, presence of confusion, blood urea, respiratory rate, blood pressure, serum creatinine (SC), C-reactive protein (CRP), white cell count (WCB), length of hospital stay (DH) and outcome of therapy.
Results: The total number of patient screened was 30, 21 males and 9 females. Median age was 69.1 years (range 42–92 years, 21 patients >65 years). Average duration of hospital stay was 16.8 days with 23.3% mortality. Positive CURB-65 criteria were: acute confusion, blood urea >8.3 mmol/l, respiratory rate >30/min, diastolic BP ⩽60 mmHg or systolic BP ⩽90 mmHg and age >65 years. Bacteria most often isolated from blood were: *S. pneumoniae* in 10 patients, *E. coli* in 4 patients, *K. pneumoniae* in 3 patients and Methicillin-sensitive *S. aureus* (MSSA) in 3 patients and *H. parainfluenzae* in 2 patients.
Conclusion: According to results CURB-65 is a good indicator of prognosis and outcome, but not of the length of hospital stay in bacteraemic CAP patients. Bacteraemic CAP in elderly could be presented even with mild clinical course and good prognosis. We found higher levels of serum creatinine a predictor of higher mortality.

Table 1: Correlation between CURB-65 criteria, age, serum creatinine, CRP, WBC and average duration of hospital stay and mortality in bacteraemic patients admitted due to CAP

No. of positive CURB criteria	No. of patients	Median SC (umol/L)	Median CRP (mg/L)	Median WBC ($\times 10^9$/L)	Median DH (days)	Mortality (%)
0	1	60.0	143.9	12.8	36.0	0
1	4	49.8	34.1	16.0	12.0	0
2	14	93.7	181.5	11.7	21.3	14
3	8	122.9	213.2	13.8	12.0	13
4	2	157.0	342.0	11.8	19.0	50
5	1	355.0	434.9	5.2	1	100

R2280 Changes in the detection of *C. trachomatis* in a female population in Greece during the last 4 years

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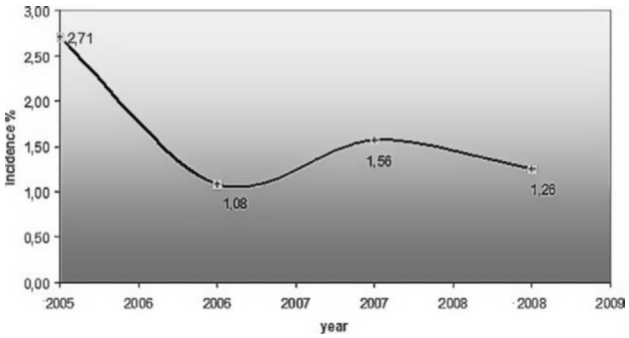
Background: Our purpose was to measure and compare the annual incidence of *Chlamydia trachomatis* infection in an urban population of women of reproductive age during the last 4 years in Athens Greece and report the relevant findings.
Material and Methods: In total, 6554 endocervical samples of unique women that attended our hospital outpatients' gynaecological department during the last 4 years (1/1/2005–31/12/2008) were included in the study. The molecular method used for the detection of *C. trachomatis* was SDA (strand displacement amplification) BD PROBE TEC TM. Patients were also stratified by age, pregnancy, previous miscarriages, symptoms and nationality.



Results: The figure shows the respective incidences of *C. trachomatis* in our study population during 2005–2008. The observed decrease is in the magnitude of >50% between 2005 and 2008 ($P < 0.05$). To our knowledge there exists no known factor for the significant decrease in the measured incidence of *C. trachomatis* infection of women in Athens, Greece.
Conclusion: In light of the recent discovery of new plasmid mutants of *C. trachomatis* that evade detection by standard molecular methods in Sweden, we believe that attention is warranted for the observed decrease in our own study population. Although the BD PROBE TEC assay is believed not to be affected by the presence of mutant strains, there is an evident need for the investigation of factors that have attributed to this change.

R2281 Invasive pneumococcal disease in adults: clinical forms and spectrum of serotypes

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Objectives: Invasive pneumococcal disease (IPD), mainly pneumonia and purulent meningitis, occur in all age groups with the highest frequency in elderly and children. Despite being less common than pneumococcal upper respiratory tract infections, they may be severe and even lethal. The aim of our study was to find out the frequency of clinical forms, clinical outcome and spectrum of *Streptococcus pneumoniae* serotypes causing invasive infections.
Methods: A retrospective epidemiological study of adult patients with IPD was carried out at four departments (Infectious Diseases, Pneumology, Internal Medicine, Surgery) of the University Hospital Bulovka in Prague, Czech Republic, in years 2000–2007. Patients older than 18 years with symptoms of acute infection were included if *S. pneumoniae* was isolated in blood or CSF culture. All strains were subsequently serotyped.
Results: Ninety-four patients with IPD were included in the study, 40 females and 54 males with the age range 19–87 years and median age of 57 years. The highest frequency of IPD (32 pts, 34%) was recorded in the age group of 50–64 years. The clinical forms were pneumonia (54 pts, 57%), meningitis (37 pts, 40%), bacteraemia (1 patient), primary peritonitis (1 patient), and septic arthritis (1 patient). IPD had lethal outcome in 23 cases (25%) with the highest case fatality ratio 50% in the age group of 65 years and older. Case fatality ratio was 20% in pneumonia (11/54) and 30% in meningitis (11/37). The most frequent serotypes were 3 (12 pts), 4 (11 pts), 8 (10 pts), 1 (7 pts), 7F (7 pts), 14 (6 pts), 6A (6 pts). Only 17% of isolated *S. pneumoniae* serotypes would not be covered with the 23-valent polysacharide vaccine.
Conclusion: IPD may have severe course with even lethal outcome mainly in elderly. The results support the importance of active surveillance of IPD with regard to both the spectrum of available vaccines and detection of population at risk.
Acknowledgement: The study was partially supported by the grant IGA MZ CR NR/8770–3.

R2282 Incidence of *Ureaplasma urealyticum* in women with chronic urinary symptoms

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Objective: The aim of the present study was to evaluate the incidence of *U. urealyticum* in women presenting with chronic urinary symptoms at our hospital.
Methods: We studied 153 consecutive women referred to our academic hospital for chronic voiding symptoms who underwent urologic evaluation between January 2007 and March 2008. Patients with UTI were excluded from the study. Only women with no prior cultures or assays for the identification of mycoplasmas were enrolled in the study while subjects receiving antibiotics within the previous month were not included. Samples from the urethra, vagina and cervix were

obtained from all women. In order to identify aerobic microorganisms samples were inoculated on blood agar, MacConkey agar, Chapman and Sabouraud agar followed by incubation at 37ºC for 24 hours, whereas anaerobic cultures were carried out on Wilkins-Chalgren agar at 37ºC for 48 hours. The automated system VITEK 2 (BioMerieux, France) was used for the identification of isolated strains while for the identification of *U. urealyticum*, the *Mycoplasma* IST 2 (BioMerieux, Marcy l'Etoile, France).

Results: The median age of the women studied was 51.7 years (range 24–70). *U. urealyticum* was detected from at least one site in 81 (52.9%) women. In particular, 26 (32.1%) women had positive cultures for *U. urealyticum* from one site (12 from urethra, 5 from vagina and 9 from cervix) while in 55 (67.9%) women, positive cultures were obtained from all 3 sites. In 27 (17.7%) cases, *U. urealyticum* was the only pathogen isolated, in 10 (6.5%) patients *U. urealyticum* was associated with *Streptococcus agalactiae*, in 10 (6.5%) with *Enterococcus faecalis*, in 9 (5.9%) with *Staphylococcus aureus*, in 6 (3.9%) with *Pseudomonas aeruginosa*, in 3 (2.0%) with *Klebsiella pneumoniae*, in 10 (6.5%) with *Gardnerella vaginalis* and 6 (3.9%) with *Candida* spp. Twenty four (15.7%) women had positive cultures only for *Enterococcus faecalis* (17) or *S. agalactiae* (7) while 16 (10.4%) had Gram-negative rods and 5 *G. vaginalis*. Finally, in 27 (17.7%) patients no pathogens could be isolated.

Conclusions: A high incidence of *U. urealyticum* was observed in our study group. Therefore, it is important to test all patients with chronic urinary symptoms for the presence of this microorganism.

R2283 Asymptomatic bacteriuria may assist urinary tract infections?

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Objectives: Urinary tract infections (UTI) have a great mortality and morbidity rate at geriatric patients. Asymptomatic bacteriuria (ASB) is frequent in elderly and even more prevalent in residents of long-term care facilities. Relation between ASB and UTI is not clear yet. Resistant bacteria can frequently cause persistant ASB.

Methods: Study group was consist of residents of three big nursery homes in Izmir. Patient selection criteria were; being over 65 years old who had not urinary symptoms but had not urinary catheterisation at least for fifteen days and not had given any antibacterial thearpy in one week. Two urine samples were taken with interval of 24–48 hours by clean catch technique. The patients who has ASB, were observed for UTI for six months. New urinary cultures were made for asympthomatic bacteriuric patients at thirth month. All isolates were investigated for resistance of ampicilline, aztreonam, amoxicilline-kalvulonate, ceftriaxone, cefotaxime, ceftazidime, amicasine, norfloxacine, ciprofloxacine, trimethoprime-sulfamethoxazole.

Results: Prevalence of ASB was 24.1% (146/606). *Escherichia coli* (87/146, 59.5%), *Klebsiella* pneumonia (23/146, 15.7%), *Klebsiella oxytoca* (18/146, 12.3%) were the first three pathogens of ASB. *Proteus mirabilis* (15/146, 10.4%) and *Proteus vulgaris* (3/146, 2.1%) were the other agents. 117 of all 146 ASB have trimethoprim-sulfamethoxazole resistance (80.1%). Ciprofloxacine resistant *E. coli* (21, 24.1%) and *K. pneumonia* (3, 13%) were persistant at thirth and sixth month. All patients were followed up for six months and UTI was not observed.

Conclusion: Four types of infections occur most often among geriatrics: urinary tract, respiratory tract, skin and soft tissue, and gastrointestinal tract. UTI is the most important clinical stiuation among these, because identifying symptoms and underlying conditions that physicians use to determine treatment for a UTI may assist the long-term care facility (LTCF) nurse in optimal use of time and resources. ASB is one of the critical points and must determine UTI correlation.

R2284 Comparison of bacterial isolates in acute post-traumatic tibial fracture infection and chronic posttraumatic tibial osteomyelitis

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Objectives: Acute posttraumatic infection of opened tibial fracture and chronic posttraumatic tibial osteomyelitis are serious illnesses compromising limb function and their treatment usually constitutes a difficult medical problem. The aim of the study is to present the microbiological findings in patients of both groups and find out if the bacterial isolates significantly differ.

Methods: All patients were treated by radical debridement and muscle flap transfer. Perioperatively sampled tissue was routinely cultured. The study included patients with positive culture as well as negative culture but clinically apparent purulency. The χ^2 test of independence contingency table was used to compare categorial data. The level of significance for the test was set at 5%.

Results: Between January 1, 2002 and September 30, 2007, 52 patients were included in the study group. There were 10 women and 42 men with a mean age of 44 years (range, 10 to 67 years). In 36 patients with acute posttraumatic tibial fracture infection Gram-positive bacteria, Gram-negative bacteria and mixed flora was isolated in 19, 9 and 7 patients, respectively. Two patients had got negative cultures. In 16 patients with chronic posttraumatic tibial osteomyelitis Gram-positive bacteria, Gram-negative bacteria and mixed flora was isolated in 6, 4 and 3 patients, respectively. Three patients had got negative cultures. The differencies between groups did not prove statistical significance.

Conclusion: Nonsignificant differencies between groups of bacterial isolates in both diagnoses, even though they cannot exclude haematogenous seeding as a cause of chronic posttraumatic tibial osteomyelitis, rather support the theory of reactivated long-persisting bacterial infection in the site of injury.

R2285 Real practice of antimicrobials use in the treatment of sexually transmitted diseases in Russia

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Objectives: Prompt treatment of sexually transmitted diseases (STDs) is an essential component of their control. So, we aimed to reveal real practice of treatment of bacterial STDs throughout Russia.

Methods: A multicentre retrospective study was conducted in 10 different cities of Russia. Clinical records of adult patients treated for bacterial STDs during Jan 2007–Dec 2007 were collected.

Results: The data on 1250 patients (61% male, 39% female, mean age 28.8±9.2) with: early syphilis (n = 341), uncomplicated gonococcal (n = 309), chlamydial (n = 310), mycoplasmic (n = 137) and ureaplasmic (n = 153) infection were analyzed. Overall 1567 disease orientated prescriptions were registered. Antimicrobials accounted for 1352 (86.3%) and were represented by six groups: macrolides (30%), cephalosporins I-III (22%), penicillins (19.5%), tetracyclines (15.1%), fluoroquinolones (9.5%), aminoglycosides (4%). Early syphilis was commonly treated by benzathine penicillin (38.4%), procaine penicillin (28.3%), ceftriaxone (26.9%) and penicillin G (5.5%); gonococcal infection – by ceftriaxone (57.5%), spectinomycin (9.3%), doxycycline (7.2%), azithromycin (5.1%); chlamydial infection – by azithromycin (28.2%), doxycycline (22.2%), claritromycin (14.9%), josamycin (11.1%), ofloxacin (7.9%); mycoplasmic and ureaplasmic infection – by doxycycline (31.8%), josamycin (21.3%), azithromycin (13.1%), claritromycin (12.8%), levofloxacin (5.6%). Other agents accounted for less than 5% each. Administered therapy went in conflict with modern national guidelines in 28.2% of patients, international guidelines – in 24.2%. Among correctly chosen agents only 24% and

11%, respectively, were used in recommended course doses (higher dose – 69.1% and 83.7%, lower – 6.9% and 5.3%).
Conclusion: Relatively low compliance to national and international guidelines as well as existing tendency to use antibiotics in higher then recommended course doses require administrative actions to change this situation throughout our country.

Lyme borreliosis, toxoplasmosis

R2286 Lyme borreliosis: descriptive study of 151 patients followed up in a university hospital, Lyon (France)

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Objectives: To describe epidemiological, clinical and serological features of patients with a positive screening test for Lyme borreliosis (LB), followed in the Hospital University of Lyon.
Methods: A descriptive study taking place among patients with positive or equivocal test Enzygmost® (Dade-Berhing) for IgM and/or IgG antibodies against *Borrelia*, between May and December 2007. All the sera were tested with immunoblot (IB) Euroline® (EuroImmun, BioAdvance) daily used in the laboratory. In each case, we studied the all patient clinical file.
Results: 151 patients were included. 33 (22%) of them were diagnosed as LB, 10 (6.6%) were likely LB and 101 (67%) were non confirmed. 7 files were not avalaible.
The median age was 54 years, range (4; 91). The sex ratio M/F was 1.2. Tick bite was reported in 27% (n = 41) and 48% (n = 16) patients among the population studied and positive LB patients respectively. Previous LB occurred in 12% (n = 18) and 18% (n = 6). Neurological complications, especially facial paralysis and meningoradiculitis, were the most frequent reported in 45% (n = 68) and 48% (n = 16) of cases. Specific antibiotherapy was prescribed in 19% (n = 19) of negative LB patients.
Among confirmed LB patients, 83% and 61% were IB positive to IgG and IgM antibodies against *Borrelia*, respectively. IB were positive in 38% (n = 38) among negative LB patients.
Conclusion: Epidemiological criteria of population studied was similar to french population LB. *Borrelia garinii* predominates in the western part of Europe which might explain the high frequency of neurological complications. LB diagnosis remained difficult and serological test results might always be interpreted in relation to the clinical findings.

R2287 Evaluation of two line immunoassays for the detection of *Borrelia* antibodies

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Objectives: Lyme borreliosis is a multisystemic disease with diverse clinical manifestations transmitted to humans by ticks. Clinical diagnosis is difficult. In routine practice a two step test strategy is recommended for the diagnosis of the disease. Several confirmation tests are available. We evaluated the performance characteristics of two different line immunoassays.
Methods: 62 sera submitted to the Laboratory Medicine unit of the University Hospitals Leuven were analyzed for the presence of *Borrelia* IgM and IgG antibodies with two line immunoassays: recomLine *Borrelia* (Mikrogen) and *Borrelia* Europe LINE (VIROTECH) and with an immunoblot assay recomBlot *Borrelia* (Mikrogen) as reference method. All tests were performed on the auto LIA II instrument (Innogenetics). Sensitivity was evaluated on 33 preselected sera from patients with Lyme borreliosis based on clinical symptoms and the presence of *Borrelia* IgM and/or IgG with recomBlot *Borrelia* assay (8 erythema migrans, 10 arthritis, 15 neuroborreliosis). Specificity was evaluated on 29 sera (10 Epstein Barr virus IgM positive sera, 9 rheumatoid factor positive sera and 10 sera positive for rapid plasma reagin antibodies).
Results: Specificity of both IgM line assays was 89.7%. 2/29 false positive IgM results were from patients with a primary EBV infection. Cross reaction due to EBV infection can be detected by the Europe LINE assay due to the presence of the EBV-VCA gp125 antigen on the strip. One IgG positive result was obtained with both line assays. This was probably due to a previous *Borrelia* infection in a rheumatoid factor positive patient. Sensitivity for both line assays was 97% if we combine IgM and IgG test results. IgG were present in 7/8 (87.5%) of erythema migrans sera with both line assay compared to 1/8 (12.5%) sera that was IgG positive with the recomBlot assay.
Conclusion: The performance characteristics of the two *Borrelia* antibody confirmation line immunoassays, recomLine *Borrelia* and *Borrelia* Europe LINE, were very good and similar in this evaluation. From early stage sera it seems that the sensitivity of both IgG line assays is higher compared to the Mikrogen IgG recomBlot assay.

Antimicrobial clinical trials

R2288 In vitro susceptibility and pathogen prevalence data in complicated skin and skin-structure infections: results of the RELIEF study

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Objectives: The choice of antimicrobial therapy in complicated skin and skin structure infections (cSSSIs) is complex due to the potential diversity of the pathogens present. In particular, cSSSIs are characterised by a high prevalence of *Staphylococcus aureus*, including methicillin (oxacillin [OXA])-resistant *S. aureus* (MRSA) both in hospital and community settings. Furthermore, in recent years, serious cSSSIs caused by multi-drug resistant pathogens – including non-fermentive Gram-negative bacilli and Enterobacteriaceae – have become more common. This has created the need for additional therapeutic agents such as broad spectrum fluoroquinolones. In the present study, we evaluated the antimicrobial activity of moxifloxacin (MXF) and commonly used antimicrobial agents against recent clinical bacterial isolates collected in the RELIEF study.

Table: MIC_{50}/MIC_{90} (mg/L) for pathogens obtained from patients with cSSSIs

Organism	N	MXF	PIP/TAZ	AMC	OXA	CRO	CFZ	GEN	ERT	MET
Gram-positive bacteria										
MSSA	368	0.03/ 0.06	1/ 2	0.5/ 1	0.25/ 0.5	4/ 4	8/ 8	0.5/ 1	0.12/ 0.25	–
MRSA	48	2/ 4	16/ >128	8/ >32	>8/ >8	64/ >64	64/ >64	>32/ >32	2/ >32	–
S. pyogenes	73	0.12/ 0.25	⩽0.25/ ⩽0.25	⩽0.06/ ⩽0.06	–	⩽0.06/ ⩽0.06	0.12/ 0.25	8/ 8	⩽0.015/ ⩽0.015	–
S. agalactiae	50	0.12/ 0.25	0.5/ 0.5	0.12/ 0.12	–	0.12/ 0.12	0.5/ 1	32/ 32	0.06/ 0.06	–
S. equisimilis	31	0.12/ 0.25	⩽0.25/ ⩽0.25	⩽0.06/ ⩽0.06	–	⩽0.06/ ⩽0.06	0.25/ 0.25	8/ >32	0.03/ 0.03	–
E. faecalis	135	0.25/ 16	4/ 8	1/ 1	–	>64/ >64	>64/ >64	16/ >32	8/ 8	–
Gram-negative bacteria										
E. coli	126	0.06/ 16	2/ 4	4/ 16	–	⩽0.06/ 0.12	0.12/ 0.25	0.5/ 2	⩽0.015/ ⩽0.015	–
Non-ESBL	119	0.06/ 2	2/ 4	4/ 16	–	⩽0.06/ ⩽0.06	0.12/ 0.25	0.5/ 2	⩽0.015/ ⩽0.015	–
ESBL	7	16/ >32	2/ 16	8/ 16	–	>64/ >64	1/ 16	16/ >32	0.03/ 0.06	–
P. aeruginosa	21	8/ >32	16/ >128	>32/ >32	–	>64/ >64	8/ 64	8/ >32	8/ >32	–
A. baumannii	23	2/ 8	64/ >128	>32/ >32	–	>64/ >64	32/ >64	>32/ >32	4/ 16	–
Anaerobes										
B. fragilis	51	0.5/ 2	0.25/ 1	–	–	–	–	–	–	1/ 1

– denotes not tested.

Methods: RELIEF was a double-dummy, double-blind, randomised controlled trial conducted from 2007 to 2008 in 15 countries, most in Europe. Acceptable culture specimens included skin biopsy, curettage of the wound base after debridement, tissue or bone biopsy, aspiration of purulent secretions including leading-edge needle aspiration. Clinically significant isolates (n = 1120) were recovered for processing from 603/803 patients in the ITT population who had at least one organism

at isolated at baseline. Minimum inhibitory concentrations (MIC) values for MXF and amoxicillin/clavulanic acid (AMC), ceftazidime (CFZ), ceftriaxone (CRO), ertapenem (ERT), gentamicin (GEN), metronidazole (MET), OXA and piperacillin/tazobactam (PIP/TAZ) were determined using validated reference broth microdilution panels. Testing, incubation and MIC interpretation were performed according to CLSI guidelines.
Results: The most frequently isolated pathogens (found in ⩾20 patients) were *S. aureus* (416/1120, 37.1%) of which 12% were methicillin resistant, *Enterococcus faecalis* (135/1120, 12.1%), *E. coli* (126/1120, 11.3%), *Streptococcus pyogenes* (73/1120, 6.5%), *Bacteroides fragilis* (50/1120, 4.5%), *Streptococcus agalactiae* (50/1120, 4.4%), *Streptococcus equisimilis* (31/1120, 2.8%), *Acinetobacter baumannii* (23/1120, 2.1%) and *Pseudomonas aeruginosa* (21/1120, 1.9%). MICs for the pathogens vs antimicrobial agents are shown in the Table.
Conclusion: MXF demonstrated good in vitro activity against most cSSSI pathogens. MXF MIC values were comparable with those of antimicrobial agents commonly used for the treatment of cSSSIs.

R2289 Clinical outcomes with daptomycin used as first-line therapy and after administration of other antibiotics for *Staphylococcus aureus* infection

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Objectives: Daptomycin exhibits in vitro concentration-dependent bactericidal activity against most clinically relevant Gram-positive pathogens. The primary objective of this study was to compare clinical outcomes of patients treated with daptomycin as first-line therapy versus after prior antibiotic therapy, and secondarily to identify potential risk factors of poor outcome of daptomycin therapy.
Methods: This was a retrospective cohort study using data from the Cubicin Outcomes Registry and Experience database (2005–2007). Clinical outcomes of patients treated for *Staphylococcus aureus* infections with daptomycin were evaluated (cure, improvement, failure). The effects of relevant patient characteristics on clinical outcome were examined using univariate and multivariate statistical analyses.
Results: Of 1227 clinically evaluable patients, 250 (20%) received daptomycin as first-line therapy and 977 (80%) after other prior antibiotics. Clinical success was reported for 1140 (93%) of 1227 patients. Univariate analysis identified 10 patient factors associated with outcome: 7 with clinical failure (ICU, reduced renal function, diabetes, concomitant antibiotics, bacteraemia, endocarditis, and prior vancomycin failure) and 3 with clinical success, cure or improvement (outpatient daptomycin and complicated and uncomplicated skin and skin structure infections). Only the presence of infective endocarditis (OR 2.56), bacteraemia (OR 1.77), reduced renal function (OR 1.78), and diabetes (OR 1.79) were identified by stepwise multivariate analysis as risk factors independently associated with clinical failure of daptomycin therapy.
Conclusion: Daptomycin is equally effective as first-line therapy and subsequent therapy following other antibiotics, including vancomycin, in the treatment of *S. aureus* infections. Further prospective and pharmacoeconomic studies to evaluate daptomycin as salvage or first-line therapy are needed.

Paediatric infections

R2290 The role of *Staphylococcus aureus* in atopic dermatitis

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Objectives: Atopic dermatitis is a chronic inflammatory skin disease associated with colonisation of the skin with *Staphylococcus aureus* known to produce toxins with superantigen activity. A correlation between the severity of the eczema and colonisation with *S. aureus* has been demonstrated and it has been determined that bacterial colonisation is an important factor aggravating skin lesions. Recent studies revealed that nasal carriage of *S. aureus* (NCSA) could be an important risk factor for subsequent infection including MRSA (methicillin-resistant *S. aureus*). The aim of this study was to determine the prevalence of *S. aureus* and MRSA in the lesional and nonlesional skin, and in the anterior vestibule of the nose in patients affected by atopic dermatitis. In addition we also examined the relationship between *S. aureus* skin lesion and nasal colonisation, the production of toxins and the presence of nasal colonisation in patient's cohabitants.
Methods: Nasal and skin (lesional and nonlesional) swabs cultures for bacterial isolation were obtained from 94 children affected by atopic dermatitis. Nasal swabs were taken from 15 patients' cohabitants. *S. aureus* strains were tested for emo-agglutination passive reverse (Oxoid, UK) for detecting the toxins SEA, SEB, SEC, SED and TSST-1.
Results: *S. aureus* colonisation prevalence estimates were 36% in the lesional skin of atopic dermatitis patients, in the same group of patients the percentage of *S. aureus* in the anterior vestibule of the nose was 94.4%. In the group of study we found the presence of MRSA with a prevalence of 7.9% in the lesional skin and 3% in the nose. 65% of *S. aureus* strains isolated from patients releases staphylococcal enterotoxins type A, B, C, D (SEA-SEB-SEC-SED) and/or TSST-1. We observed that all positive patients to *S. aureus* had at least one positive cohabitant and that the presence and the kind of enterotoxins in strains isolated from cohabitants coincide to 100% with those of the patients.
Conclusion: This data focus the importance of the nasal carriage as risk factor for the development of skin lesions in atopic dermatitis patients. It's important to redefine the exact pathogenetic role of *S. aureus* for a better therapeutic strategy as *S. aureus*, in particular strains producing toxins, could be considered a trigger or aggravating atopic dermatitis. In the current diagnostic practice is appropriate to include the research of *S. aureus* in the patients' family cohabitants.

R2291 Procalcitonin as prognostic marker of infection complicated in newborn with congenital heart disease

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Objectives: Recent data indicate that prenatal latent infections in newborns with congenital heart disease (CHD) may increase risk of postoperative infectious complications. Early recognitions of this process can improve the results of surgery. The aim of our study was to estimate the diagnostic value of procalcitonin (PCT) as a predictor of infectious complications in newborn with CHD.
Methods: Blood plasma samples from 37 newborns with CHD on the first week of life (mean age 4 (1–6) days, median weight 3.23 (1.98–4.24) kg) were taken before surgery. PCT concentrations were measured by immunoluminometric method (PCT sensitive LIA, B.R.A.H.M.S Aktiengesellschaft GmbH, Germany). The data were compared by Mann-Whitney U-test and Wilcoxon matched pairs test, p-value of <0.05 was considered statistically significant. The data are expressed as median and 25th and 75th percentiles.

Table 1

	PCT, ng/ml			
	Vaginal delivery			Operative delivery
	preterm (n = 3)	at term (n = 26)	40–42 weeks (n = 3)	at term (n = 5)
Median	0.6	0.33	0.05	0.85
Percentiles	0.25–1.21	0.21–1.0	0.05–0.39	0.34–0.89

Results: All newborns were without clinical signs of infections, but twenty-five of 37 patients (68%) had evidence of higher levels of PCT before surgery.
There was no correlation between newborns age and levels of PCT ($r = -0.31$, $p = 0.7$). The PCT levels were higher in newborns of 12–48 hours of life in comparison with newborns aged after 48 hours of life but the data weren't statistically significant (0.8 (0.3–1.4, n = 10)

vs. 0.31 (0.15–0.9, n = 27, respectively). The PCT levels didn't depends statistically significant from the gestation and type of delivery (table 1). The type of congenital heart disease, antibiotic prophylaxis in first hours of life, prescription of prostaglandin E weren't impacted on PCT level also.

Meanwhile, retrospective study have shown statistically significant difference between PCT level before surgery in group of newborn with postoperative infection complicated and without it (0.66 (0.38–2.03) vs 0.3 (0.24–0.89) ng/ml, respectively, p = 0.03). Predictor point of infection complications were 0.345 ng/ml cut-off PCT value before surgery with sensitivity and specificity 83% and 62% respectively.

Conclusions: Our data suggest that 68% of newborns with congenital heart disease have latent microbial process. The plasma concentration of PCT may serve as a prognostic marker in newborn before repair of congenital cardiac disease. Cut-off value of PCT 0.345 ng/ml may be used as "look-out level" of infetion before cardiac surgery in newborns.

R2292 Murine typhus in a paediatric hospital in Greece

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Objectives: The aim of this study is to present the clinical and epidemiological characteristics of *Rickettsia typhi* infection in children, during the last five years.

Methods: Between October 2003 and October 2008, 496 children under 15 years of age, admitted to our hospital with fever, were tested for *Rickettsia typhi* infection. For the serological diagnosis an indirect immunoflorescence assay was used.

Results: Serological diagnosis of acute *Rickettsia typhi* infection (IgM antibody titers of 64 or higher and IgG antibody titers of 256 or higher to typhus group antigen) was found in 17 children (9 boys and 8 girls, median age 11 years). The most frequent manifestations of rickettsial disease were fever (100%) and rash (41%). Rash appeared as maculopapular or erythematous. Splenomegaly (29%) and lymphadenopathy (18%) were noted less frequently. Abnormal laboratory findings included mild elevation of liver enzymes, anaemia, leukopenia and thrombocytopenia. Most of the cases presented at fall (88%). The residence of the majority of the children was in the provinve or in the suburban area of Attica (76%).

Conclusion: Murine typhus is rare in children, usually presented in fall months with fever and rash. Serological tests in symptomatic children from endemic areas should be performed.

Internet and electronic resources

R2293 Evaluating the user experience of the Sealife Semantic Web browsers

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Objectives: The purpose of the Sealife project, launched in 2006, is to create browsers that use the Semantic Web to create a faster, better focussed, and richer search experience for infectious disease professionals, whose time is at a premium.[1] From December 2008 to March 2009, a user-centric evaluation of the Sealife browsers was undertaken.

The evaluation required each user to carry out specific search tasks using either the National Electronic Library of Infection (NeLI, http://www.neli.org.uk) or PubMed, to determine whether the Sealife browsers enhanced their search experience compared with those websites' search engines alone. For example, some users were asked to find the answer to the question "What percentage of viral encephalitis and meningitis cases in the UK are of undetermined aetiology?" This project will demonstrate how Semantic Web browsers can help users to find answers to their questions more quickly and more efficiently.

Methods: The evaluation was carried out online, in a workshop, and through focus groups. Participants were recruited through the NeLI and National Resource for Infection Control (NRIC, http://www.nric.org.uk/) mailing lists, and the NeLI Advisory Board.

The methods used were as follows:

- Pre- and post-evaluation questionnaires
- Questionnaires following each evaluation task
- Web server log analysis
- Interviews

The scenarios were assigned to each user according to browser compatibility and/or at random. Each user worked through half of the tasks using a Sealife browser, and half without. The tasks were always presented in the same order, but were counterbalanced such that some users began with a Sealife browser and ended without, and other users vice versa.

Results: This ongoing study thus far has 24 users, a majority of whom have reported that the Sealife browsers increased the usability, ease, and speed of their search experience, and increased the relevance and findability of information.

Full results will be analyzed in February and March and will be presented at the conference.

Conclusions: Preliminary results from our survey demonstrated that Semantic Web browsers can enhance the speed, relevance, and richness of the existing Web search experience for the life scientist.

Reference(s)

[1] "Sealife: A Semantic Grid Browser for the Life Sciences applied to the study of Infectious Diseases", ICT for Health – Resource Book of eHealth Projects – FP6
http://www.biotec.tu-dresden.de/sealife/download/sealifeLeaflet.pdf